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MODIFICATION OF THE ZEISS-PULFRICH PHOTOMETER FOR THE MEASUREMENT OF 90° SCATTERING AND DISSYMMETRY[1]

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Received November 14, 1951

Light scattering techniques have been applied with considerable success to the study of properties of colloidal solutions and solutions of macromolecules. The molecular weight of the solute can be determined with accuracy comparable to or greater than other methods commonly used for this purpose; furthermore, the required measurements can be made with rapidity and comparative ease.[3]

Debye (2) has shown that the turbidity is related to the molecular weight of the solute molecule by the expression

$$\tau = \frac{32\,\pi^3\,n_o^2\,(\partial n/\partial c)^2\,c}{3N_o\,\lambda^4\,(1/M + 2\beta/RT\,c)}$$

Here n_o is the refractive index of the solvent; c is the concentration of solute in grams per milliliter; N_o is Avagadro's number; λ is the wave length of the incident light; M is the molecular weight of the solute, β is a constant and R and T have their usual significance. The constant H may be defined as

$$H = \frac{32\,\pi^3\,n_o^2\,(\partial n/\partial c)^2}{3\,n_o\,\lambda^4}$$

and may be considered constant under a given set of experimental conditions; thus

$$HC/\tau = \frac{1}{M} + \frac{2\beta}{RT}\,c$$

The turbidity is determined at various concentrations and Hc/τ is plotted against c. The resulting plot is a straight line whose intercept at c = O gives the reciprocal of the molecular weight.

The molecular weight obtained in this fashion is valid only if the solute molecules are isotropic and small compared with the wave length of the light used. When these conditions are not met the turbidity must

[1] Journal Paper No. J-2022 of the Iowa Agricultural Experiment Station. Project 978. Supported in part by a gift from Swift and Co.

[2] Present address: Carbide and Carbon Chemicals Co., Oak Ridge, Tenn.

[3] Light scattering has been the subject of several excellent recent reviews: H. Mark, *Frontiers in Chemistry 5*, 121(1948); G. Oster, *Chem. Rev. 43*, 319(1948); B. H. Zimm, R. S. Stein, and P. Doty, *Polymer Bull.* 1, 90(1945); J. T. Edsall and W. B. Dandliker, *Fortschr. Chem. Forsch. 2*, 1(1951).

be corrected by suitable factors which may be evaluated by the measurement of dissymmetry and depolarization. The latter error can probably be assumed negligible in the case of most protein solutions. Dissymmetry of the scattering envelope, determined by making measurements of the scattered intensity at two angles equally spaced about the 90° position (for example at 45° and 135°), cannot be assumed negligible *a priori.* If any dimension of the scattering particle exceeds about 1/20 the wave length of the radiation used, this effect becomes significant, and indeed measurement of the dissymmetry provides an excellent means of approximating the long dimension of the scattering unit.

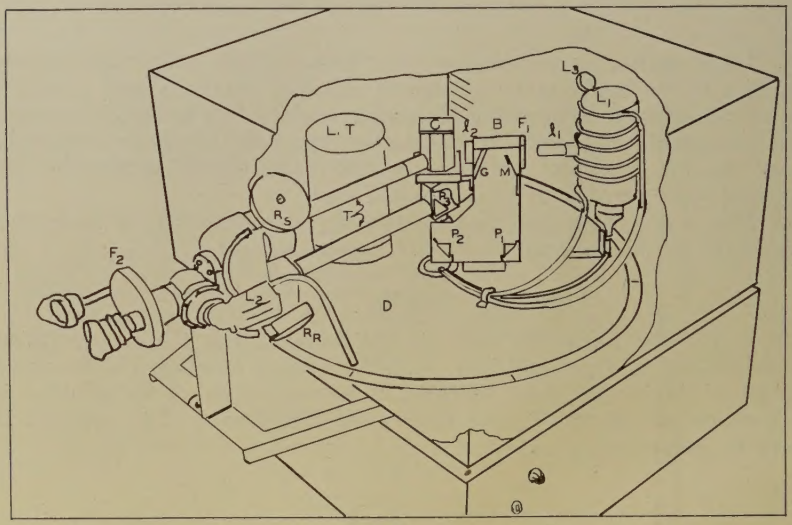

FIG. 1a. Cut-away drawing of light scattering photometer showing essential features.

It was desirable to construct a simple inexpensive instrument which would permit the operator to measure scattered light intensities as a function of angle. A number of instruments have been described (1, 7) and have been shown to give satisfactory results. The use of the optical system of a Zeiss-Pulfrich photometer in the manner described by Stein and Doty (7) offered the most promise for our purposes. It was necessary, however, to modify their design to permit measuring scattered intensities at angles other than 90°.

APPARATUS

Figure *1a* is a sketch of the instrument identifying the various essential features. (L_1) is a water cooled low pressure AH4 mercury arc to serve as light source. Lens mounting (l_1) holds a lens couple to

render the light from the arc parallel. The light passes next through a filter (F_1) which isolates the selected Hg line. The filter also serves as one window of the light-tight, dust-tight optical box (B). The main beam of light passes through the inclined glass plate (G) and through the lens (1_2) which serves the double function of bringing the light to a focus in the scattering cell and acting as the second window of the box. The light then passes through the scattering cell (C) and into the light trap (L.T.). The cell table holding the scattering cell is mounted to the

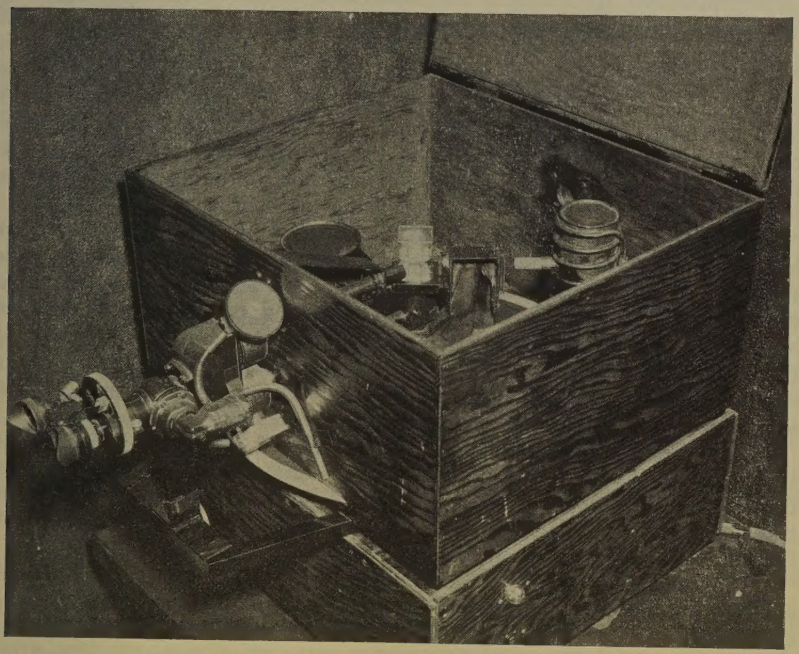

FIG. 1b. Photograph of the light scattering photometer.

optical box so that the cell position is fixed with respect to the incident beam.

A small fraction of the incident light is reflected from the inclined glass plate (G), then from the small mirror (M) to the right angle prism (P_1), again at the prism (P_2) to (P_3) which is mounted in a stationary box so that the light reflected from it always passes into the lower tube (T), and thence to the photometer. The optical box is so mounted that the reference beam passing from (P_2) to (P_3) is on the axis of rotation of the optical box.

The tubes (T) pass the light from the scattering cell and the reference beam to the Zeiss-Pulfrich photometer. Ground glass plates are inserted into the path of the reference beam to render the reference

field uniform in intensity and to decrease the intensity of the reference beam to the same order of magnitude as that of the scattered light from the cell. The photometer is mounted so that it is inclined 90° to the position usually used. This permits both fields of the instrument to be illuminated by light coming directly from secondary sources located on the axis of rotation of the optical box. The intensity of the light coming from the reference beam through the lower tube can be matched with that coming from the scattering cell through the upper tube. The match is accomplished by rotating the calibrated drums (R_S and R_R). The drums control diaphram openings which are proportional to the dial readings.

The light source, optical box, cell table, and light trap are mounted on the large magnesium disc (D). The disc is mounted at the center on a machined bearing and supported near the circumference by four rollers mounted under the disc. The disc and assembly can be rotated so that the scattered light can be read at any desired angle from —145° to +145° with respect to the direction of the incident beam.

The disc, light source, optical box, cell table, and light trap are enclosed in a box to prevent dust and external light from entering. The tubes (T) extend through the front of the box and pass the light from the scattering cell and the reference beam to the photometer. A portion of the magnesium disc extends outside of the box through a slit near the bottom, allowing rotation of the disc and assembly from the outside of the box. The inside of the box and all parts except filter, prisms, lenses, and mirror are painted black to eliminate reflections of stray light.

The electrical leads for the AH4 light and the rubber tubing for the leads to the cooling coil around the lamp housing enter the box through the hollow bearing at the center of the magnesium disc. The transformer, switch, and pilot light are mounted in a second box which serves also as a stand for the instrument.

Dials R_R and R_S and the setting of the disc can be read with the aid of illumination originating from a 6 watt lamp completely housed at (L_2). Lucite rods leading from the lamp housing "pipe" the light to the point needed. This greatly minimizes the illumination required for the readings and lessens eye strain due to adjustment of the eye to different intensity levels. The lucite rods are encased in rubber tubing; thus the only light escaping from the housing and assembly is from the ends of the rods, at the scale positions.

The light scattering cell used was designed by B. A. Brice and made by Pyrocell Manufacturing Co. It is a six sided sinter-fused glass cell so constructed that the cell contents can be viewed at 0°, 45°, 90°, and 135° normally to the faces of the cell. Dissymmetry and turbidity can thus be determined using the same cell. A minimum of 30 ml. of solution is required. Smaller cells may be used for turbidity measurements by using an adapter for the cell table.

A permanent stationary lamp (L_3) was mounted in the back of the box opposite the end of the upper tube leading to the photometer. This light could be connected to a 6 volt storage battery and was used to

check the uniformity of the intensity of the reference beam as a function of angle.

The Zeiss-Pulfrich photometer is designed for use with light filters located in the filter turret (F_2). Providing the sample in the scattering cell shows no fluorescence, the filters in the turret may be used instead of using filters at F_1. It was found that the same results were obtained for ovalbumin with the filter in either position. This was not true, however, for all materials tested. In the case of amyloheptaose, for example, a tenfold change in intensity was noted with the same filter at F_1 and at F_2. The filter was used in position F_1 for the work reported in this paper.

It was found convenient to use the instrument in the darkroom. By working in the darkroom, light intensities as low as two to three times that of the solvent could be measured with reasonable accuracy.

OPERATION AND PERFORMANCE

Measurements are made in the light scattering instrument by placing the scattering cell in position, rotating the magnesium disc to the desired angle (45°, 90°, 135°), setting the upper dial of the photometer to a selected value (usually 100), and then matching the light in the two fields of the photometer by rotating the lower dial. The lower dial reading is then proportional to the intensity of the scattered light and, hence, to the turbidity of the sample by the relationship:

$$\tau = \tau(\text{Standard}) \times \frac{\text{Lower dial reading for sample}}{\text{Lower dial reading for standard}}$$

The standard and the sample must be measured with the upper dial at the same setting.

The secondary reference standard used was a lucite block $1\frac{3}{4}'' \times 1\frac{3}{4}'' \times 2\frac{1}{2}''$. It was prepared by machining the faces of a larger block to this size, and then polishing the surfaces with soft grit free paper. Only 90° scattering was used and the block was always placed in the cell table with the faces in the same relative positions. By taking the average of a number of readings the turbidity of the plastic secondary standard could be checked with an accuracy of better than one per cent.

The intensity of the reference beam is independent of the angle at which a reading is made. This was demonstrated as follows: A small 6 v. light was mounted in the back wall of the box and connected to a standard lead storage battery. Suitable filters (Cenco No. 4 and No. 5) and ground glass plates were placed before the upper tube leading to the photometer to render light of the proper shade and intensity to match that of the reference beam. A mask was placed over the exit of the optical box to prevent any possible interference from the regular beam of the mercury lamp. Readings were made of the matched fields at 45°, 90°, and 135°. The averages of six readings for each position were in agreement, and the average deviation from the mean for the readings for any set was less than 1 per cent, (30.4 ± 0.3).

CALIBRATION

The defining equation of turbidity, $I = I_0 e^{-\tau l}$, can be used to determine the turbidity of a solution directly. In order for τ in the above expression to truly represent turbidity the scattering solution must meet the following requirements: scattering must be the only phenomenon contributing to the diminution of the intensity of the transmitted beam; the scattering particles must be small with respect to the wave length of the light used; the colloidal solution should be stable; and the solution must exhibit sufficient scattering to permit a relatively large difference between I and I_0.

Dr. John T. Edsall[4] of Harvard has used a solution of colloidal silica ("Ludox") which met the necessary requirements. He kindly forwarded us a sample of the silica preparation. Tests on the sample were not as satisfactory as hoped. Dissymmetry measurements indicated that the sample was not monodisperse and measurements were not time independent. However, by applying the same clarification procedures as were used for the protein preparations (see below), samples were obtained giving low dissymmetries and consistent turbidities. Subsequently a sample of Ludox colloidal silica, 30 per cent Si O_2, was obtained from H. H. Snyder through the courtesy of E. I. du Pont de Nemours and Co. The tests on this sample proved completely satisfactory. It is suspected that the sample received from Dr. Edsall was modified in transit by possible freezing in the sub-zero temperatures occurring at that time.

Ten ml. of the Ludox colloidal silica sample were diluted to 100 ml., placed in two 50 ml. centrifuge tubes, and centrifuged at high speed (20,000g) for 20 minutes. The upper 20 ml. from each tube were transferred to enough double-distilled centrifuged water to give a solution containing approximately 0.3 per cent colloidal silica. The resulting solution was transferred to a 50 cm. Beckman absorption cell and the intensity of the transmitted light I was measured with the Beckman D. U. spectrophotometer using the special adapter for the 50 cm. cell. The intensity of the light transmitted through double-distilled centrifuged water (I_0) was also measured. The turbidity of the Si O_2 solution is then given by

$$\tau = \frac{2.30}{50} \log \frac{I_0}{I}$$

The intensity of the light scattered at 90° by this suspension was then measured with the light scattering photometer and compared with that from the plastic reference standard. (The values obtained on the sample received from Dr. Edsall were obtained in the same fashion except that the solutions measured were prepared from the 5 per cent stock solution received. The final concentrations were approximately 0.25 and 0.07 per cent silica and it was necessary to filter, or filter and centrifuge the sample to reduce the dissymmetry and increase the stability of the solutions.)

[4] Private communication.

The values obtained on the silica samples are given below:

Source	τ *SI* O_2 *Sol'n.*	τ *Plastic St.*
Edsall (filtered)	9.06×10^{-3}	1.31×10^{-4}
Edsall (filtered and centrifuged)	2.31×10^{-3}	1.29×10^{-4}
Du Pont (centrifuged)	10.77×10^{-3}	1.27×10^{-4}

The average value of 1.29×10^{-4} was taken as the turbidity of the plastic reference standard in all of the work reported in this paper.

Three other methods of calibration were tried; namely, calibration using the known scattering power of pure carbon disulfide, calibration using a solution of a standard polystyrene preparation [5] dissolved in toluene, and calibration using the reflectivity of a magnesium carbonate surface. The first two of these methods require evaluation and use of correction factors arising from the difference between the refractive index of water and the indices of the organic solutions. These correction factors depend on instrument constants which are difficult to evaluate for the instrument described here. The calibration with the magnesium carbonate surface is subject to criticism on several counts. Consideration of these factors led the authors to acceptance of the calibration using colloidal silica as most accurate.

EXPERIMENTAL

Measurement of Specific Refractive Increment

In order to evaluate the constant H in the turbidity equation it is necessary to know the specific refractive increment $(\partial n/\partial c)$. Inasmuch as the change in the refractive index is proportional to the concentration, it is necessary to measure $n\text{-}n_o$ at only one concentration. The ratio $\frac{n\text{-}n_o}{c}$ then gives the specific refractive increment of the solution.

Commercial differential refractometers can be obtained or an instrument can be constructed using the principle outlined by P. P. Debye (3). Perlmann and Longsworth (6) have made a most detailed study of the specific refractive increments of some purified proteins. Their measurements were made using a hollow prism method employing the optical equipment of the electrophoresis apparatus.

The specific refractive increments used in this investigation were measured with the optical equipment of the electrophoresis apparatus in a manner similar to the method of Perlmann and Longsworth. There are, however, significant differences which must be mentioned. The cell used for measuring refractive increments in this study had two compartments. The two compartments were separated by a diagonal plate which met the front face of the cell one cm. from the bottom and at an angle of 55°. The lower portion of the cell was filled with solution and the upper compartment with solvent. The cell was sealed with a rubber gasket and placed in position on a brass rack. The rack and cell were positioned in the electrophoresis water bath fixing the

[5] Sample supplied through the courtesy of Prof. P. Debye and Dr. A. M. Bueche of Cornell University.

position of the cell with respect to the beam of light.

The narrow beam of light passing through the bottom compartment passes undeflected, but that through the upper portion is deflected downward in passing from the solution to the solvent. The downward deflection is proportional to $n-n_0$.

The use of this type of cell has the advantage that successive readings are independent of any possible change in the refractive index of the water in the constant temperature bath; furthermore, measurements of n can be made for systems in which the solvent has a refractive index appreciably different from water.

The cylindrical lens method of obtaining the schlieren pattern is well known and will not be described here. The method has been found

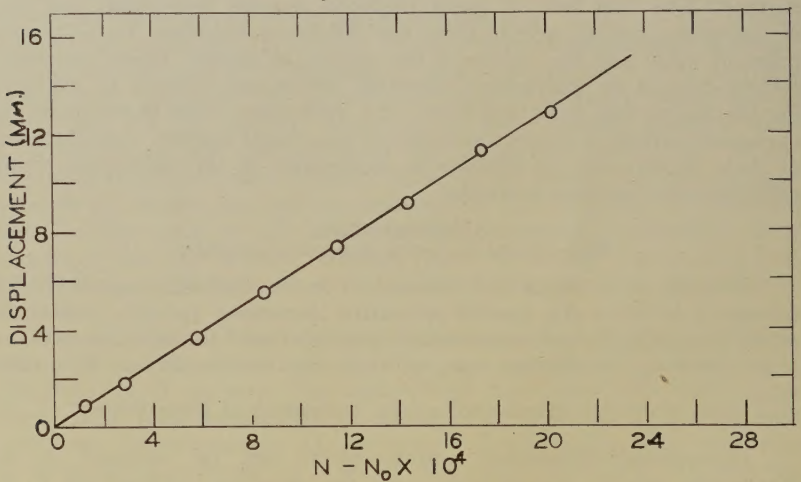

FIG. 2. Plot of displacement (in millimeters) of the light beam in the cylindrical lens optical system as a function of the refractive index increment between standard solution of sucrose and solvent (water).

entirely satisfactory for obtaining patterns from which the refractive increment can be determined. It has the advantage over the schlieren scanning method in that the displacement can be followed with a traveling telescope, thus requiring no photographic manipulation.

The traveling telescope was mounted directly on the end of the electrophoresis optical bench in the focal plane of the light from the cylindrical lens. The rotating drum on the telescope could be read to 0.02 mm., which corresponds to a difference in refractive index of less than 0.000003. For a solution containing one gram of ovalbumin per 100 ml. of solution, this corresponds to 3 parts in 1876.

A calibration curve was obtained from the cell and instrument using sucrose solutions of known concentration and refractive indices. Water was used as the solvent. The curve obtained is shown in Figure 2. It

is to be noted that a straight line was obtained, indicating that corrections are not required due to imperfections of the cylindrical lens. The displacement can be photographed if desired. Figure 3 is a series of photographs taken of the pattern produced by solutions containing 0.0, 0.1, 0.2, 0.4, 0.6, 0.8, 1.0, 1.2, and 1.4 per cent sucrose. The displacement is proportional to the concentration.

The refractive increment for ovalbumin is 0.188 when c is expressed

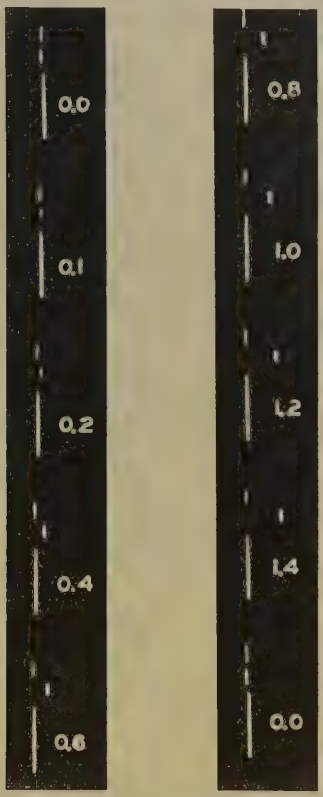

FIG. 3. Photographs of the patterns obtained with standard sucrose solutions of varying concentration (as shown).

in gms. per ml. This value was used for all work with ovalbumin reported in this dissertation. The following values were obtained for the other substances employed in this work: bovine serum albumin 0.194, lysozyme 0.187, and amyloheptaose 0.149.

Clarification of Solutions for Turbidity Measurements

A combination of filtration and centrifugation was found more

effective for clarification than either alone. The dissolved samples were filtered first through a Hormann pressure filter using a number eight "Steriflow" bacteriological filter pad plus "Celite" filter aid. The Celite filter aid had been washed, dried, and mixed to insure freedom from soluble components and also uniformity. The filtrate from the Hormann filter was discharged directly into a specially constructed all-glass filter containing a "fine" sintered glass disc. The filtrate from the sintered glass filter was discharged into 50 c.c. centrifuge tubes. The samples were then centrifuged for one hour at top speed in a Sorvall SS-1 high speed

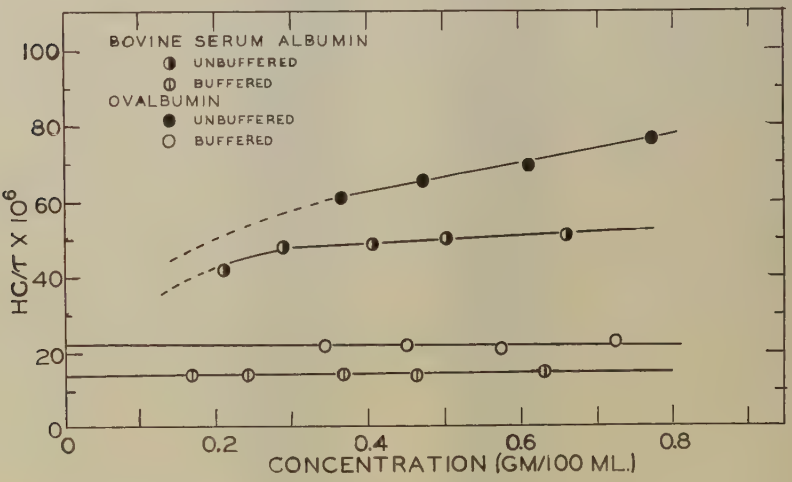

FIG. 4. Plot of HC/τ vs. concentration for bovine serum albumin and ovalbumin.

angle centrifuge. The solvent for making dilutions was treated in a similar fashion.

Protein concentrations were determined using a modified Pregl micro — Kjeldahl procedure.

RESULTS

The molecular weights of three crystalline proteins, namely bovine serum albumin, ovalbumin, and lysozyme, were determined. The molecular weight of amyloheptaose was also determined using the light scattering photometer.

Solutions of the three proteins were prepared in identical fashion. Enough of the crystalline protein[6] was taken to give the clarified solution

[6] *Bovine serum albumin*: Received through the courtesy of Dr. Walter L. Hughes, Jr. of Harvard Medical School. *Ovalbumin*: Three times recrystallized by Dr. E. Samsa of these laboratories. Lot K. *Lysozyme*: Three times recrystallized by Dr. E. Samsa.

a concentration of approximately 0.8 gm/100 ml., and was dissolved in water or in phosphate buffer[7] giving a *p*H of 7.85.

Figures 4 and 5 show the Hc/τ vs. c curves for the proteins. It is to be noted that the curves for the buffered samples in each case lead to values at (Hc/τ) which are the reciprocal of the molecular weight for the protein. In the case of the essentially salt-free solutions the apparent molecular weights are much too low for reasons which have been discussed by Edsall *et al.* (4). The apparent molecular weights obtained for the three proteins are given in Table 1 along with values obtained by other investigators.

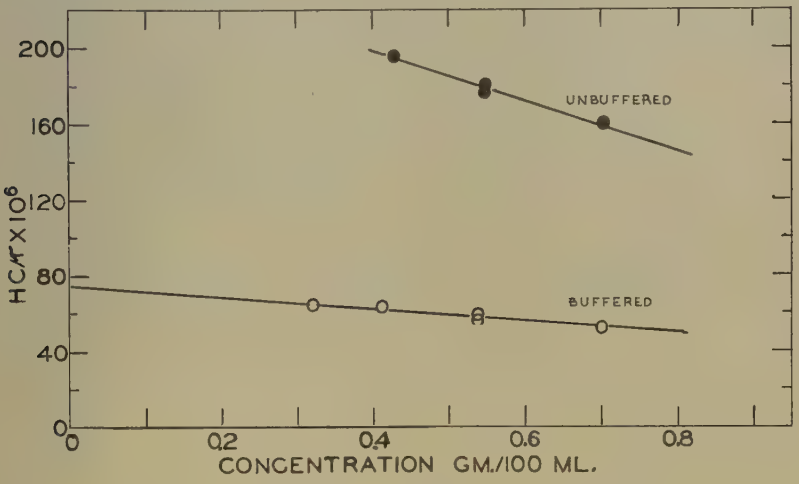

FIG. 5. Plot of HC/τ vs. concentration for lysozyme.

The amyloheptaose used was furnished through the courtesy of Drs. D. French and J. Pazur of these laboratories. It is a water soluble material of high purity (5). It was prepared by acid hydrolysis of the crystalline Schardinger β-dextrin under conditions which gave predominantly the seven glucose unit amylose. Separation and reprecipitation gave a material whose chemical and physical properties agree well with those to be expected for amyloheptaose. Any trace impurities should be of low molecular weight and therefore should not interfere with the determination of the molecular weight by light scattering.

The Hc/τ vs. concentration curve for amyloheptaose is shown in Figure 6 and the resulting molecular weight is given in Table 1 along with the theoretical molecular weight calculated from the formula of the compound, C_{42} H_{72} O_{36}. The solution for turbidity measurements was

[7] The phosphate buffer had the following composition: K_2H PO_4 0.0324M, KH_2PO_4 .0036M, NaCl 0.1M. The ionic strength of the buffer is 0.20.

prepared by dissolving the amyloheptaose in double-distilled water. The solution was boiled 10 minutes to remove any alcohol. The cooled sample, clarified by filtration and centrifugation in the usual manner, was transferred to the scattering cell and a dilution series made using filtered and centrifuged double-distilled water for making the dilutions. The concentration of the solution was determined by measuring the optical rotation of the filtered, centrifuged sample with a polaroscope. The

TABLE 1

COMPARISON OF THE MOLECULAR WEIGHTS OF BOVINE SERUM ALBUMIN, OVALBUMIN, LYSOZYME, AND AMYLOHEPTAOSE WITH THOSE OBTAINED BY OTHER INVESTIGATORS

Sample and Method	Molecular Weight
Bovine Serum Albumin	
Osmotic pressure	69,000*
Light scattering	72,500†
Light scattering	74,500–84,000‡
This investigation	72,500
Ovalbumin	
Osmotic pressure	42,000–46,000§
Ultracentrifuge	40,500–44,000§
Light scattering	45,700†
This investigation	46,200
Lysozyme	
X-ray diffraction	13,900‖
Light scattering	14,700†
This investigation	13,400
Amyloheptaose	
Theoretical	1152
This investigation	1050

* Scatchard, Batchelder, and Brown, *J. Am. Chem. Soc.*, *68* 2320(1946).
† Brice, Halwer, and Speiser, *J. Optical Soc. Am.*, *40*, 768(1950).
‡ Edsall, Edelhoch, Lontie, and Morrison, *J. Am. Chem. Soc. 72*, 4641(1950).
§ Cohn and Edsall, "Proteins, Amino Acids and Peptides" N.Y. Reinhold Publishing Corp. (1943).
‖ Palmer, Ballantyne, and Galvin, *J. Am. Chem. Soc. 70*, 906(1948).

optical rotation of amyloheptaose for the D line of sodium was taken as 179.6. This value was calculated by French *et al.* (5) using Freudenberg's equation and the constants for starch.

Measurements of scattered intensity at 45° and 135° were made routinely on all solutions along with the 90° readings. From the known physical properties of the proteins investigated it can be concluded that none should have any dimension greater than 100 to 200 A, and hence none should show any appreciable dissymmetry. It was found rather difficult to obtain proteins with no dissymmetry and it was concluded that the dissymmetry is an excellent measure of the effectiveness of the clarification procedure used. The procedure finally adopted and outlined above was selected in large part on this basis and indeed the

ratio of scattered intensity at 45° to that at 135° approached unity, to within a few hundredths of a unit, for all of the solutions reported on above.

SUMMARY

1. A visual light scattering photometer is described. The instrument adapts the optical system of a Zeiss-Pulfrich photometer to the measurement of scattered light intensities at angles of from —145° to +145° with respect to the incident beam. This permits measurement of not only 90° scattering but dissymmetry.

2. Results are given for measurement of the molecular weight of three purified proteins and for the polysaccharide, amyloheptaose. A molecular weight of 72,500 was obtained for bovine serum albumin, 46,200 for ovalbumin, 13,400 for lysozyme, and 1,050 for amyloheptaose. These values are all in reasonable agreement with molecular weights reported by other investigators.

3. The dissymmetry of scattering was found to be a useful criterion of the effectiveness of clarification with the proteins studied. A combination of filtration and contrifugation was found most effective for the preparation of clarified solutions. Solutions clarified in this way gave substantially no dissymmetry.

4. The refractive increment of the materials investigated was measured by a modification of the Longsworth electrophoresis technique. The method makes use of a two compartment cell for producing a deflection of the light beam proportional to the refractive increment of the solution. The deflection is measured by a traveling telescope mounted in the focal plane of the cylindrical lens.

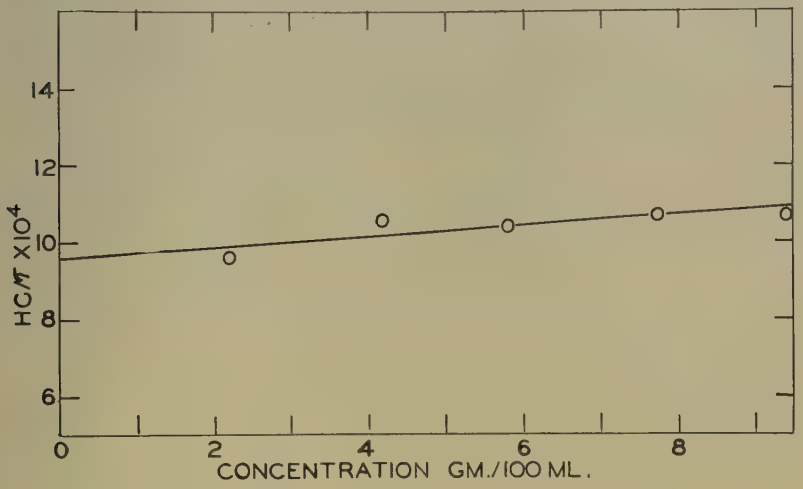

FIG. 6. Plot of HC/τ vs. concentration for amyloheptaose.

LITERATURE CITED

1. Brice, B. A., M. Halwer, and R. Speiser
1950. Photoelectric light-scattering photometer for determining high molecular weights. Jour. Opt. Soc., Am. 40, 768.
2. Debye, P.
1947. Molecular-weight determination by light scattering. Jour. Phys. and Coll. Chem. 51, 18.
3. Debye, P. P.
1948. A photoelectric instrument for light-scattering measurements and a differential refractometer. In Robinson, High Polymer Physics, Chemical Publishing Co., New York.
4. Edsall, J. T., H. Edelhoch, R. Lontie, and P. R. Morrison
1950. Light scattering in solutions of serum albumin: effects of charge and ionic strength. Jour. Am. Chem. Soc. 72, 4641.
5. French, D., M. L. Levine, and J. H. Pazur
1949. Schardinger dextrins. II. Preparation and properties of amyloheptaose. Jour. Am. Chem. Soc. 71, 356.
6. Perlmann, G. E. and L. G. Longsworth
1948. The specific refractive increment of some purified proteins. Jour. Am. Chem. Soc. 70, 2719.
7. Stein, R. S., and P. Doty
1946. A light scattering investigation of cellulose acetate. Jour. Am. Chem. Soc. 68, 159.

THE COCCINELLIDAE (COLEOPTERA) OF THE UPPER MISSISSIPPI BASIN[1]

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The following identification keys and annotated list of the family *Coccinellidae* as it occurs in the Upper Mississippi Basin are presented with the hope of partially bridging the gap of some fifty years that has elapsed between the monograph of Casey (2) and the present time. Although, in the interim the works of Leng (18, 19, 20, 21), Blatchley (1), Stehr (27), Dobzhansky (7,8), and Chapin (4) have appeared, these authors dealt with either a single genus of the family for the United States or with the family as it occurs in a single state. The need for adequate identification keys and distribution records for the midwestern Coccinellids has been apparent for some time. The keys of Casey, Horn, Leng, and Blatchley, standard identification references for the occasional student of the family, although excellent for the time and material at hand, are at some variance and often serve to confuse rather than clarify. The author has drawn freely from these sources and acknowledges indebtedness for their use.

The term Upper Mississippi Basin (Fig. 40) as used in this paper includes the states of Ohio, Indiana, Illinois, Michigan, Wisconsin, Minnesota, Iowa, Missouri, and the states of Kansas and Nebraska east of the one hundredth meridian. The author fully realizes that in the strict geographic sense goodly portions of the northern states included above are not in the drainage basin of the Mississippi River. However, the term serves to identify a study area which is a compact geographic unit containing a somewhat common fauna. The inclusion of the northern states in their entirety brings into the study a few boreal species which are not common to the area as a whole. Also, the southern portions of Ohio, Indiana, Illinois, and Missouri contain species which are typically southern in distribution. It is felt that species distribution in the area studied is somewhat indicative of distribution in other regions of the United States.

The principal collections containing *Coccinellidae* were visited both in the east and in the study area. The author gratefully acknowledges the kindness of the following institutions and their curators for permission to study collections in their care and for the generous loan of material:

[1] A dissertation submitted to the Graduate Faculty in partial fulfillment of the requirements for the degree of Doctor of Philosophy.

United States National Museum, Academy of Natural Science of Philadelphia, American Museum of Natural History, Museum of Comparative Zoology, University of Michigan Museum of Zoology, Michigan State College, Ohio State University, Purdue University, Illinois State Natural History Survey, Chicago Natural History Museum, University of Minnesota, University of Wisconsin, University of Nebraska, University of Kansas, University of Missouri, Iowa Insect Survey at Iowa Wesleyan College, and Iowa State College. In addition to these institutional collections smaller private collections or lots of specimens were made available by private collectors. Thanks are due these: Richard C. Froeschner, W. S. Craig, W. R. Enns, Geo. W. Thomas, Jean Laffoon, James A. Slater, and Harry E. Brown.

In the section dealing with the genus *Scymnus,* Missouri records are frequently indicated by collectors' initials. These may be identified by the following list:

C.W.B.—Dr. Chas. W. Bock, late physician and ardent coleopterist of St. Louis, Mo.

W.S.C.—W. S. Craig, graduate student at Iowa State College.

W.R.E.—W. R. Enns, Instructor in Entomology at the University of Missouri.

R.C.F.—R. C. Froeschner, Instructor in Zoology and Entomology at Iowa State College.

E.H.F.—Elsie Herbold Froeschner, wife of R.C. Froeschner.

C.W.W.—The author.

R.I.W.—R. I. Wakeman, Entomologist, California Spray Chemical Company.

The members of the family Coccinellidae may be recognized by the following description:

Form convex-oval or round to elongate with the convexity less pronounced; upper surface glabrous or pubescent; head usually deeply inserted in the prothorax; maxillary palpi 4-segmented with a large securiform (rarely conical) terminal segment; antennae short (except *Coccidula*), 11-segmented ending in a 3 segmented club; abdomen usually with 5 ventral segments, the first widest with curved coxal lines; metasternum large, frequently with a depression for the reception of the middle legs; legs short; tarsi apparently 3-segmented (4-segmented with the true third segment fused to the base of the last), first and second dilated and spongy beneath; claws appendiculate, toothed or simple.

KEYS TO THE SUBFAMILIES, TRIBES, GENERA, AND SPECIES OF COCCINELLIDAE OF THE UPPER MISSISSIPPI BASIN

1. Upper surface of the body pubescent or glabrous: mandibles bifid or simple at the tips, basal tooth present . Subfamily COCCINELLINAE Ganglbauer (p. 16)
   Upper surface of the body always pubescent; mandibles with several teeth at the tips, basal tooth always wanting ..... Subfamily EPILACHNINAE Ganglbauer (p. 25)

Subfamily COCCINELLINAE Ganglbauer

1. Upper surface of the body glabrous or apparently so 2
   Upper surface of the body pubescent 7

2. Body elongate and loosely formed; femora extending well beyond the sides of the body ..... Tribe *Hippodamiini* Costa (p. 22)
Body rounded, convex and compact 3
3. Epistoma broadly dilated so as to subdivide the eyes; form round and very convex ..... Tribe *Chilocorini* Costa (p. 24)
Epistoma not broadly dilated 4
4. Genital segment developed so as to resemble a true visible sixth ventral abdominal segment ..... Tribe *Hyperaspidini* Costa (p. 17)
Genital segment not appearing as a sixth abdominal segment 5
5. Prosternum greatly expanded affording protection to the mouth; epipleurae with an abrupt pit at middle of the length ..... Tribe *Oeneini* Casey (p. 22)
Prosternum not expanded; epipleural depression, if present, not an abrupt pit 6
6. Pronotum broadly reflexed at the sides; length of the body 3 mm. or less.... ..... Tribe *Psylloborini* Casey (p. 22)
Pronotum narrowly reflexed at the sides; length of the body greater than 3 mm. ..... Tribe *Coccinellini* Weise (p. 23)
7. Body elongate; prothorax narrowed at the base; antennae as long as the prothorax ..... Tribe *Coccidulini* Costa (p. 22)
Body round or oval; prothorax not narrowed at the base; antennae shorter than the prothorax 8
8. Pubescence conspicuous and profuse ..... Tribe *Seymnini* Costa (p. 19)
Pubescence sparse; body apparently glabrous and shining; punctures of the elytra and thorax each with a single, fine, erect hair .... Tribe *Microweiseini* Leng (p. 19)

Tribe *Hyperaspidini** Costa

1. Anterior tibiae with an acute external edge and an external spine about two-thirds from the base ..... III *Brachyacantha* Chevrolat
Anterior tibiae slender, without an external spine 2
2. Epipleurae foveate for the reception of the hind femora .... I *Hyperaspis* Redtenbacher
Epipleurae without foveae for the reception of the hind femora .... II *Hyperaspidius* Crotch

I *Hyperaspis* Redtenbacher

1. Elytra with a spot at the humeral angle or with a marginal vitta 2
Elytra without a spot at the humeral angle or a marginal vitta. 14
2. Elytra with a spot at the humeral angle 3
Elytra with a marginal vitta from the humeral angle 5
3. Elytra with a basal spot near the suture ..... 14 *disconotata Muls.*
Elytra without a basal spot near the suture 4
4. Elytra with a rounded spot on the disc and a transversely oval apical spot ..... 16 *octavia* Csy.
Elytra with a suffuse subapical dash, often with a suffuse discal dash .... 21 *moerens* Lec.
5. Lateral vitta of the elytra reaching nearly to the apex 6
Lateral vitta of the elytra reaching only one-half or three-quarters of the length 11
6. Elytra with an oblong oval pale spot on the disc ..... 15 *undulata* (Say)
Elytra without a pale spot or vitta on the disc 7
7. Elytra with only a marginal vitta ..... 12 *fimbriolata Melsh*
Elytra with both marginal and discal vitta 8
8. Marginal vitta of the elytra expanded at the middle, joining the discal vitta to form a broad, pale discal area ..... 22 *bolteri* Lec.
Marginal vitta not expanded at the middle 9
9. Marginal and discal vitta joined at the apex of the elytra ..... 18 *annexa* Lec.
Marginal and discal vitta not confluent at the apex of the elytra 10
10. Pronotum variable in color and pattern, dull yellow to dark brown with irregular pale margins ..... 20 *brunnescens Dobz.*
Pronotum black with yellow lateral margins longer than wide .... 19 *quadrivittata Lec.*

* According to best authority a tribe based on the genus *Hyperaspis* should be spelled *Hyperaspidini*, and should a tribe be based on *Hyperaspidius* the spelling would be *Hyperaspidiini.*

11. Elytra with a discal pale spot 12
Elytra without a discal pale spot ..... 13 *dissoluta coloradana* Csy.
12. Marginal vitta not over one-half the length of the elytra .. 2 *lateralis* Muls.
Marginal vitta three-fifths the length of the elytra 13
13. Discal spot of elytra elongate oval ..... 3 *lugubris* (Rand.)
Discal spot of elytra rounded; form elongate ..... 17 *punctata* Lec.
14. Elytra each with a single pale spot 15
Elytra each with two or three pale spots 20
15. Pale spot of elytra subapical, nearer lateral margin than suture .....
..... 11 *bigeminata* (Rand.)
Pale spot of the elytra on the disc 16
16. Discal pale spot extending nearly to the lateral margin of the elytra; elytral margin narrowly black ..... 8 *lewisi* Cr.
Discal pale spot separated by its width or more from the lateral margin of the elytra 17
17. Discal pale spot of the elytra equidistant from the lateral margin and suture . 18
Discal pale spot of the elytra closer to the margin than the suture, placed in front of the middle of the length ..... 9 *bicentralis major* Dobz.
18. Pronotum with subquadrate yellow spots at the lateral margins 19
Pronotum narrowly yellow at the lateral margins or completely black .....
..... 5 *binotata* (Say)
19. Center of the discal pale spot of the elytra on or slightly in front of the middle of the length, round, transverse, or extending into a point posteriorly .....
..... 10 *rivularis* Dobz.
Center of the discal pale spot of the elytra distinctly posterior to the middle of the length ..... 7 *pinorum* Csy.
20. Elytra with two pale spots ..... 6 *signata* (Ol.)
Elytra with three pale spots 21
21. Marginal spot of the elytra near or at the middle of the length .....
..... 1 *pratensis* Lec.
Marginal spot of the elytra opposite the apical spot ..... 4 *proba* (Say)

II *Hyperaspidius* Crotch

1. Elytra with at least the suture black 2
Elytra and thorax entirely pale flavo-testaceous; head slightly darker than the body; form oblong; elytra slightly truncate at the tips . 24 *transfugatus* Csy.
2. Elytra yellow with the suture broadly black and a black vitta over the callus; form oval, convex ..... 25 *militaris* (Lec.)
Elytra variable in maculation, dark with narrow pale vittae or pale with broad dark vittae 3
3. Head and pronotum pale, the latter irregularly dark at the base or with a bilobed dark area extending to the middle of the disc 4
Head and thorax black, the latter with the lateral margins yellow; elytra black with pale lateral and discal vittae ..... 23 (♀) *vittigerus* (Lec.)
4. Elytra pale, lateral margin very thinly black, a black vitta extending from or near the base nearly to the apex where it turns toward the suture and joins the black area ..... 23 (♂) *vittigerus* (Lec.)
Elytra black with a lateral yellow vitta from the humeral angle to near the appex, wider at the apex than at the base ..... 23 (♂) *vittigerus* (Lec.)

III *Brachyacantha* Chevrolat

1. Basal and humeral pale spots of the elytra entirely lacking; third abdominal segment of males bicuspid; large, length 5 mm. or greater .. 26 *dentipes* (Fab.)
At least the basal spot present near the suture 2
2. Form oval or nearly round 3
Form elongate-oval, sides of the elytra subparallel 4
3. Pale spots of the elytra five in number, large, and rounded .... 29 *bolli* Cr.
Pale spots of the elytra two or three in number, rarely with four or five spots in which case the humeral and discal spots are small and indistinct .....
..... 30 *4-punctata* Melsh.
4. Size larger, normally 3.5–4.5 mm. in length; maculation of the elytra greatly variable, black with five pale spots of the typical pattern to pale with the suture and spots at the callus and subapex black ..... 27 *ursina* (Fab.)

Size small, less than 2.5 mm. in length, elytral pattern of five pale spots constant, except the humeral and basal occasionally confluent .... 28 *felina* (Fab.)

Tribe Microweiseini Leng

I *Microweisea Cockerell*

1. Form oval, convex, body tapering behind the middle of the length; color shining black or brown ..... 31 *misella* (Lec.)
   Form oval, convex, body rounded behind; color castaneous ..... 32 *marginata* (Lec.)

Tribe Scymnini Costa

1. Prosternum deflexed so as to partly or completely cover the mouthparts .. 2
   Prosternum not deflexed anteriorly, mouthparts visible 3
2. Size large, length 4 mm. or more; mouthparts completely covered by the prosternum ..... V *Cryptolaemus* Mulsant
   Size small, length less than 2.5 mm.; mouthparts only partly covered by the prosternum ..... I *Stethorus* Weise
3. Anterior coxae narrowly separated; thorax about as wide as base of elytra .. 4
   Anterior coxae widely separated; thorax distinctly narrower than base of elytra, anterior margin of pronotum deeply emarginate ..... IV *Cephaloscymnus* Crotch
4. Clypeus and frons greatly expanded in front of the eyes, prothorax in repose concealing the anterior coxae and prosternum ..... III *Nephaspis* Casey
   Clypeus and frons not or only slightly expanded in front of eyes; anterior coxae and prosternum not hidden by the prothorax ... II *Scymnus Kugelann*

I *Stethorus* Weise

1. Body black, sparsely pilose; legs pale ..... 33 *punctum* (Lec.)

II *Scymnus* Kugelann

1. Metacoxal lines recurved toward the base of the abdomen 2
   Metacoxal lines not recurved toward the base of the abdomen 3
2. Metacoxal arc incomplete, line not reaching the base of the first abdominal segment..... (A) Subgenus *Scymnus* s. str. Kugelann
   Metacoxal are complete, formed by the line reaching cephalad to the base of the first abdominal segment ..... (B) Subgenus *Pullus* Mulsant
3. Metacoxal lines not reaching the posterior margin of the first abdominal segment, continued laterally parallel to the posterior margin ..... (C) Subgenus *Nephus* Mulsant
   Metacoxal line joining the posterior margin of the first abdominal segment .. .. (D) Subgenus *Diomus* Mulsant

(A) Subgenus *Scymnus* Kugelann

1. Elytra black, apex broadly tipped with reddish brown ..... 34 *americanus* Mulsant
   Elytra black, each with a large oval red spot in front of the middle of the disc ..... 35 *circumspectus* Horn

(B) Subgenus *Pullus* Mulsant

1. First ventral abdominal segment of males not modified at middle, punctures and pubescence uniform in size and distribution throughout middle area of segment 2
   First ventral abdominal segment of males modified at middle, having an elevated area, a tubercle or a glabrous area; the latter may be defined by densely set punctures and/or longer pubescence 10
2. Body rufo-testaceous above; pronotum with a basal black spot or a basal black spot confluent with a triangular black area along the suture 7
   Body black above; elytra and pronotum variously marked with pale marginal areas or with spots 3
3. Apex of elytra with a broad, clearly-defined pale area covering at least one-sixth of the length 8
   Apex of elytra black or only narrowly pale, pale area involving only the bead or a narrow adjacent portion of the elytra 4

4. Pronotum entirely black or black with the anterior angles pale 5
Pronotum entirely yellow or yellow with a small, nebulous black area before the scutellum 6
5. Pronotum entirely black; elytra black with a red spot placed just behind the middle of the disc; prosternal carinae incomplete ..... 36 *punctatus* Melsh.
Pronotum black, often with the anterior angles pale; elytra black without spots, apical margin narrowly pale; prosternal carinae variable .. 37 *nanus* Lec.
6. Pronotum yellow or reddish yellow, frequently with a small nebulous black area in front of scutellum; body elongate-oval; fifth ventral abdominal segment of males very slightly impressed, not foveate; penis of males shorter than parameres; ventral alae lacking ..... 38 *kansanus* Csy.
Pronotum always wholly yellow; body rounded-oval, convex; prosternal carinae always complete to the anterior margins; fifth ventral segment of male deeply impressed, with a rounded fovea; penis longer than parameres, ventral alae present ..... 39 *cervicalis* Muls.
7. Body entirely rufo-testaceous above except for a parabolic black spot at the base of pronotum ..... 40 *lodi* Stehr
Body rufo-testaceous with a long central black area formed by a parabolic black spot at base of pronotum confluent with a common triangular black area along the suture for three-fourths the length ..... 41 *cinctus* Lec.
8. Pronotum black with pale lateral margins or pale with a large, well-defined, parabolic, black spot at the base, extending far forward; apical pale area of elytra well defined, anterior margin arcuate 9
Pronotum yellow or reddish yellow, often with a small, ill-defined, dark area in front of scutellum; body oval; apical pale area of elytra not projecting further cephalad at the middle than margins or suture, anterior margin not clearly defined or arcuate; penis of males longer than parameres, narrowed at base ..... 42 *festatus* n. sp.
9. Form oval, convex; apical pale area of elytra involving one-fifth or a little less of the length, broadly arcuate anteriorly, reaching further along the lateral margins than along the suture; penis of males asymmetrical, apical process short ..... 43 *fraternus* Lec.
Form elongate-oval; apical pale area of elytra one-third the length of the elytra, evenly arcuate along the anterior margin, not broadly arcuate as in *fraternus* Lec., reaching further cephalad at the middle than at sides or suture; penis of males bilaterally symmetrical, broad at base, sharply pointed at apex ..... 44 *rubricaudus* Csy.
10. First ventral abdominal segment of males with a median glabrous area at posterior margin 13
First ventral segment of males without a median glabrous area 11
11. First ventral abdominal segment of males tuberculate at middle of hind margin or with an elevated median area just cephalad of the hind margin 12
First ventral abdominal segment of males with dense patches of longer pubescence on each side of midline; fifth ventral segment deeply sinuate at middle ..... 45 *brullei* Muls.
12. First ventral segment of males with a small tubercle at middle of hind margin; penis broadly rounded at middle, internal ventral lobes touching or overlapping ..... 46 *marginicollis* Mann.
First ventral segment of males with an elevated area at middle not reaching the hind margin of the segment; penis elongate, narrowed basally, internal ventral lobes widely separated ..... 47 *pulvinatus* n. sp.
13. Elytra with a large apical pale area; pronotum entirely yellow or yellow with a small, poorly-defined, black spot at base in front of scutellum ..... 48 *nemorivagus* n. sp.
Elytra not widely pale at apex; pronotum entirely black or black with pale lateral and anterior margins 14
14. Penis of males longer than parameres and ventral alae 15
Penis shorter than parameres, equal in length to or shorter than ventral alae 17
Penis shorter than parameres and ventral alae (Fig. 12) .. 49 *impunctus* n. sp.
15. Internal ventral margins of penis forming an acute angle near the apex (Fig. 26) parameres short and stubby, less than half the length of the penis, terminal hair tufts long ..... 50 *hortensis* n. sp.

Internal ventral margins of penis forming an acute angle near the base (Figs. 21 and 24) 16

16. Penis with median apical process of ventral surface blade-shaped in outline when seen from the side; parameres short and stubby (Fig. 24) ....53 *cultratus* n. sp.
Penis without a median blade-shaped process at middle of ventral surface; membrane from ventral alae attached near the rounded apex of the penis (Fig. 2) ..... 51 *puncticollis* Lec.

17. Penis equal in length to ventral alae 18
Penis shorter than ventral alae 20

18. Apex of penis formed into a broad hooked process curved ventrad (Fig. 23); terminal lobes of ventral alae broadly expanded; median glabrous area of male first ventral abdominal segment poorly defined .....52 *uncus* n. sp.
Apex of penis slender, not formed into a broad hooked process 19

19. Penis broadly bullet-shaped dorsally, developed ventrally into a slender pointed process extending well beyond the dorsal part; membrane connecting penis and ventral alae attached near base of penis (Fig. 34) .... 54 *natchezianus* Csy.
Penis angulate in outline near the apex, ventral process projecting little beyond dorsal part; membrane attaching penis and ventral alae forming a distinct angle with the penis, attached well toward the apex of penis (Fig. 31) ..... 55 *lacustris* Lec.

20. Median glabrous area of first ventral segment of males not impressed 21
Median glabrous area of first ventral of males distinctly impressed 22

21. Median glabrous area of first ventral segment of males defined by more densely set punctures and long sparse pubescence; ventral alae and penis very broad at base; parameres attached dorsally to base of ventral alae (Fig. 30) .... 56 *majusculus* n. sp.
Median glabrous area of male first ventral not defined by closely set punctures or longer or more thickly set pubescence; ventral process of penis slender, projecting well beyond the dorsal part (Fig. 27) ..... 57 *tenebrosus* Muls.

22. Median glabrous area of male first ventral abdominal segment impressed so as to appear feebly concave, broadly curved laterally or acutely triangular in shape; ventral process of penis projecting beyond dorsal part, not slender as in *tenebrosus*, rounded at apex (Fig. 28) ..... 58 *iowensis* Csy.
Median glabrous area of male first ventral deeply impressed, impression appearing as a small sunken glabrous area at posterior margin; ventral process of penis equal in length to the dorsal part (Fig. 29) . 59 *consobrinus* Lec.

(C) Subgenus *Nephus* Mulsant

1. Elytra black with pale spots or areas 2
Elytra pale or ferruginous ..... 60 *intrusus* Horn

2. Elytra each with one pale spot behind the middle of the length .... 61 *flavifrons* Melsh.
Elytra each with two large pale spots which are sometimes contiguous .... 62 *ornatus* Lec.

(D) Subgenus *Diomus* Mulsant

1. Elytra black, marked with pale spots and fasciae or with apex pale 2
Elytra pale luteo-flavate, with or without piceous vittae 7

2. Elytra black, marked with pale spots or fasciae 3
Elytra black, apex yellow 6

3. Elytra black, each with two yellow oblique fasciae 4
Elytra black, each with two yellow spots 5

4. Pale fasciae of elytra extending from the lateral margin, not reaching the suture ..... 63 *ohioensis* Stehr
Anterior fasciae of elytra extending from below the humeral angle to near the suture, reaching neither margin; posterior fasciae confluent with pale lateral margin ..... 64 *amabilis* Leconte

5. Pale spots of elytra large; anterior spot larger than posterior spot, extended toward humeral angle; sides of the body sub-parallel .... 65 *quadritaeniatus* Leconte
Pale spots of elytra small; anterior spot much smaller than posterior, placed slightly in front of middle ..... 66 *liebecki* Horn

6. Apical pale area of elytra narrow; pronotum yellow ... 67 *xanthaspis* Mulsant
Apical pale area large, at least as long as one-fourth the length of the elytra ..... 68 *terminatus* Say
7. Suture with parallel piceous vittae from base to behind middle ..... 69 *culcis* Casey
Suture without parallel vittae, body pale luteo-flavate throughout ..... 70 *aeger* Casey

III *Nephaspis* Casey

1. Pronotum yellow or yellow with a median dark area; elytra black at base and along lateral margins ..... 71 *amnicola* n. sp.

IV *Cephaloscymnus Crotch*

1. Broadly oval; upper surface black with a metallic or greenish bronze cast in strong light ..... 72 *zimmermanni* Crotch

V *Cryptolaemus* Mulsant

1. Head and thorax yellow-orange; elytra black with a blue-metallic luster, apex widely tipped with yellow-orange ..... 73 *montrouzieri* Mulsant

Tribe Oeneini Casey

1. Legs retractile within cavities of the sternum; coxae widely separated; length 1.3–1.4 mm. ..... I. *Delphastus* Casey

I *Delphastus* Casey

1. Body almost globose; elytra black shining; front femora widely expanded hiding the tibiae and tarsi in repose ..... 74 *pusillus* (Lec.)

Tribe Coccidulini

1. Body elongate; pronotum narrowed at base; antennae longer than pronotum; elytral punctures in indistinct and irregular rows ..... I *Coccidula* Gyllenhal

I *Coccidula* Gyllenhal

1. Suture without a wide black line connecting basal black area and sutural spot of elytra ..... 76 *suturalis* Weise

Tribe Psylloborini

1. Oval convex; pronotum small, sides broadly reflexed; upper surface of body grey with a pattern of brown or black spots ..... I *Psyllobora* Chev.

I *Psyllobora* Chevrolat

1. Elytra pale with 9 black spots which are often confluent ..... 77 *vigintimaculata* (Say)

Tribe Hippodamiini Costa

1. Tarsal claws simple, slender, swollen but without a subquadrate tooth at base . 2
Tarsal claws with a large subquadrate tooth at base 3
2. Side margins of elytra broadly reflexed; elytra with spots; form elongate-oval ..... I *Anisosticta* Dup.
Side margins of elytra slightly reflexed; elytra vittate; form elongate, sides of body subparallel ..... II *Macronaemia* Csy.
3. Mesepimera and metepimera black; upper surface red with black spots ..... III *Coleomegilla* Timb.
Mesepimera and metepimera white; upper surface yellow or orange, immaculate or marked with black spots ..... IV *Hippodamia* Dejean

I *Anisosticta* Duponchel

1. Elongate-oval; elytra each with nine black spots, scutellar spots common along suture for a short distance ..... 78 *bitriangularis* (Say)

II *Macronaemia* Casey

1. Elongate; elytra yellow, each with a black vitta from base to near apex, suture finely black for same distance ..... 79 *episcopalis* (Kirby)

III *Coleomegilla* Timberlake

1. Elytra red, each with six black spots, sutural spots common at scutellum and middle ..... 80 *maculata lengi* Timberlake

IV *Hippodamia* Dejean

1. Pronotum black, bordered by white, without divergent white dashes on disc 2
   Pronotum with divergent white dashes on disc 4
2. Elytra (Fig. 39) with spots ½, 1, 2, 3, 4, 5, and 6; scutellar spots (½) common at suture ..... 81 *tredecimpunctata tibialis* (Say)
   Elytra without the full complement of spots basic in the genus, spots 2 or 3 lacking 3
3. Elytral spot 2 lacking; spot 3 common at the suture and joined to spot ½ which is greatly expanded ..... 82 *americana* Crotch
   Elytral spot 3 lacking; spots 4, 5, and 6 fused, or nearly so, to form the typical parenthesis-shaped mark ..... 83 *parenthesis* (Say)
4. Elytral spot 2 lacking; spots ½, 1, and 3 fused to form a transverse sub-basal bar ..... 84 *quinquesignata* (Kirby)
   Sub-basal bar of elytra not present 5
5. Elytral spot 3 always absent; spots ½, 1, and 2 rarely present; spots 4 and 5 always confluent, spot 6 frequently confluent with 4 ..... 85 *glacialis* (Fab.)
   Elytral spot 3 present or absent; spots 4 and 5 frequently confluent, not forming the heavy transverse mark of *glacialis* 6
6. Elytral spots small and rounded, especially spots ½, 1, 2, and 3; elytra frequently immaculate ..... 86 *convergens* Guerin
   Elytral spots large, not rounded; spots 2 and 3, also 4 and 5, often confluent ..... 87 *quindecim-maculata* Mulsant

Tribe Coccinellini Weise

1. Tarsal claws cleft within above the middle ..... IX *Neomysia* Casey
   Tarsal claws with a large internal tooth at base 2
2. Elytra immaculate ..... III *Cycloneda* Crotch
   Elytra maculate 3
3. Form round, strongly convex 4
   Form rounded, somewhat elongate, not strongly convex 7
4. Epipleurae flat or only slightly concave 5
   Epipleurae concave, abruptly descending externally ..... IV *Olla* Casey
5. Pronotum with two large rectangular black spots; prosternum bicarinate ..... VII *Anisocalvia* Crotch
   Pronotum immaculate, black except front angles pale or with a median black M-shaped pattern and pale margins 6
6. Epipleurae narrowed beyond posterior one-fifth, not reaching apex of elytra; pronotum black with front angles pale ..... II *Coccinella* (Linnaeus)
   Epipleurae reaching apex of elytra; pronotum with a median black M-shaped design or rhomboidal black spot ..... VIII *Anatis* Mulsant
7. Side margins of elytra broadly reflexed; form oval ..... I *Neoharmonia* Casey
   Side margins of elytra only slightly reflexed; form elongate-oval 8
8. Metacoxal arcs almost complete; metacoxal lines distinctly recurved toward base of abdomen ..... V. *Adalia* Mulsant
   Metacoxal lines not recurved toward base of abdomen; form more elongate than *Adalia;* prosternum, episterna, and epimera white or pale yellow ..... VI *Cleis* Mulsant

I *Neoharmonia Casey*

1. Elytra red with black spots or black with a single triangular red mark on disc ..... 88 *venusta* (Melsheimer)

II *Coccinella* (Linnaeus)

1. Mesepimera and metepimera black; body broadly oval, more elongate than other species in the genus ..... 94 *hieroglyphica tricuspis* Kirby
   Mesepimera white; metepimera white, brown, or black; body broadly oval or subhemispheric 2
2. Metepimera white, often tinged with yellow 3
   Metepimera brown or black 4

3. Elytral spots connected to form three transverse black fasciae; anterior fascia common to both elytra ..... 93 *trifasciata* Linnaeus
Elytral spots not connected to form fasciae; size larger, form more broadly oval ..... 89 *movemnotata* Herbst
4. Elytra spotless ..... 90 *californica Mannerheim*
Elytra with spots 5
5. Scutellar spot expanded to join with humeral spot, forming a sub-basal bar .. .. 91 *transversoguttata* Falderman
Scutellar spot large, rounded, not joined with humeral spot to form a band or bar ..... 92 *nivicola monticola* Mulsant

III *Cycloneda* Crotch

1. Pronotal pattern without isolated white spots; penis of male pointed at apex ..... 95 *munda* (Say)
Pronotal pattern with isolated white spots placed laterally in black basal area; penis of male spoon-shaped at apex ..... 96 *sanguinea* (L.)

IV *Olla* Casey

1. Upper surface of body light grey with a pattern of spots in transverse rows ..... 97 *abdominalis* (Say)
Upper surface of body black; each elytron with a large irregluarly-shaped red or yellow spot in front of middle ..... 98 *abdominalis plagiata* Casey

V *Adalia* Mulsant

1. Elytra red, always with a single rounded black spot at middle .... 99 *bipunctata* (L.)
Elytra red or black; red forms immaculate or with one or two rows of black spots, variable; black forms always with large humeral areas and post-median spots red ..... 100 *frigida* (Schn.)

VI *Cleis* Mulsant

1. Elytra yellow with dark vittae from callus to near apex, expanded before middle and at apex, with a lateral dark spot opposite the expanded area of the vitta ..... 101 *picta* (Randall)

VII *Anisocalvia* Crotch

1. Elytra pale with black spots; pronotum with two large rectangular black spots on each side of midline ..... 102 *duodecim-maculata* (Gebler)
Elytra black with pale spots or markings, variable .... 103 *quatuordecimguttata* (L.)

VIII *Anatis* Mulsant

1. Elytra yellowish gray or reddish brown with three rows of black spots 2
Elytra brownish red or ochraeceous, immaculate but with the limb black; form very broadly rounded ..... 106 *rathvoni lecontei* Casey
2. Elytra reddish brown, with black aureolate spots; penis of males rounded, blunt at the distal end ..... 105 *ocellata mali* (Say)
Elytra yellowish gray or reddish brown, each with eight black spots; penis of males with distal end arrow-shaped or lanceolate .... 104 *quindecimpunctata* (Olivier)

IX *Neomysia* Casey

1. Pronotum with a large well-defined median dark area 2
Pronotum pale brown without maculation except feeble basal and median dark spots not well defined ..... 109 *horni* Crotch
2. Elytra yellow or brownish yellow, immaculate or with faint traces of two dark vittae uniting posteriorly ..... 107 *pullata* (Say)
Elytra yellow or brownish yellow with two distinct dark vittae joined at the base ..... 108 *randalli* Casey

Tribe Chilocorini Costa

1. Abdomen and epipleurae excavate for the reception of the hind femora 2
Abdomen and epipleurae not excavate 3

2. Tibiae dentate externally near base; hind angles of pronotum without deep rounded impressions; 5 mm. or less in length ..... II *Chilocorus* Leach
Tibiae not dentate externally; hind angles of pronotum with deep rounded impressions; size larger, greater than 5 mm. in length ..... I *Axion Mulsant*
3. Tarsal claws strongly toothed at base ..... III *Exochomus* Redtenbacher
Tarsal claws feebly toothed at base or simple ..... IV *Brumus* Mulsant

I *Axion* Mulsant

1. Elytra black, each with a large sub-basal red spot .... 111 *plagiatum* (Olivier)
Elytra black, each with a red spot near humeral angle, and a small red dash at suture forming a common sutural spot .... 110 *tripustulatum* (DeGeer)

II *Chilocorus* Leach

1. Upper surface black; each elytron with a rounded red spot at middle .....
.....112 *bivulnerus* Mulsant

III *Exochomus* Redtenbacher

1. Pronotum black; front angles and lateral margins narrowly pale; elytra orange, each with one or two black spots variable in size, sometimes so enlarged and fused as to leave the elytra largely black; length, 2.6–3.1 mm. .....
..... 113 *marginipennis* (Leconte)

IV *Brumus* Mulsant

1. Head black, pubescent; pronotum black; elytra orange lateral margin narrowly, suture widely, bordered with black; each elytron with a sub-basal and subapical black spot; length 3.8–4.4 mm. ..... 114 *davisi* (Leng)

Subfamily *Epilachninae* Ganglbauer

1. Oval, convex; tarsal claws bifid, toothed at base; sixth abdominal segment of female split; epipleurae flat; punctuation of elytra composed of both fine and coarse punctures .....I *Epilachna* Chevrolat

I *Epilachna* Chevrolat

1. Pale ground color of elytra yellow; humeral, mid-sutural and subapical elytral black spots large and rounded ..... 115 *borealis* (Fab.)
Pale ground color of elytra cupreus-brown; humeral and mid-sutural spots of elytra small ..... 116 *varivestis* Mulsant

Annotated List of Species

1. *Hyperaspis pratensis* Leconte. — Occasional specimens are seen which have all the spots larger in size with the apical spot transversely oval (*aemulator* Casey). Dobzhansky (1941) listed this form tentatively as subspecies *aemulator* Casey. The midwestern specimens seem to be minor aberrations of *pratensis* Lec. Recorded from Ohio, Indiana, Illinois, Minnesota, Iowa, Missouri, and Kansas.

2. *Hyperaspis lateralis* Mulsant. — A southwestern species seldom collected in the middle states. Collected at Wyatt, Mo., Aug. 8, 1940 by R. C. Froeschner and at Hillsboro, Kan., Oct. 21, 1943 by L. A. Calkins. The latter record was from *J. virginia* infested with *Pseudoccus juniperi*.

3. *Hyperaspis lugubris* (Rand). — Never common in the region studied, this species has been seen from Indiana, Illinois, Minnesota, Iowa, Missouri, and Kansas.

4. *Hyperaspis proba* (Say). — This species is generally distributed over the area studied, but is never collected in large numbers.

5. *Hyperaspis binotata* (Say). — A common species found in moderate abundance throughout the Upper Mississippi Basin. Stehr (1930) reports it feeding on *Lecanium* sp. Further south it is commonly collected from shrubs in early foliage.

6. *Hyperaspis signata* (Olivier). — This species is taken much less commonly than *binotata* and tends to be distributed more southerly than the latter. Recorded from Ohio, Indiana, Illinois, Wisconsin, Iowa, and Missouri.

7. *Hyperaspis pinorum* Casey. — A single record is available for this species; Scioto Co., Ohio, VI-7-44, D. J. and J. N. Knull. The species is essentially southern in distribution and we would expect it to occur infrequently in the Upper Mississippi Basin.

8. *Hyperaspis lewisi* Crotch. — Easily recognized by the very large elytral spots, this species is rare in the area. Recorded from Scioto Co., Ohio, VI-17-44, D. J. and J. N. Knull, and from Wayne and Dent counties in Missouri by R. C. Froeschner.

9. *Hyperaspis bicentralis major* Dobzhansky. — Two females collected by E. Liljeblad at Riverside and Edgebrook, Ill. and deposited in the museum of the University of Michigan answer to the description given by Dobzhansky (1941). Nothing has been seen of the typical *bicentralis* Casey in the area studied.

10. *Hyperaspis rivularis* Dobzhansky. — At the time of description Dobzhansky had specimens from Illinois, Missouri, and Kentucky. Additional specimens have been seen from Iowa and Indiana as well as several from Missouri.

11. *Hyperaspis bigeminata* (Randall). — This species is not common, but is distributed rather widely from Maine to Florida, and southwest to Texas. During the present study it has been recorded from Indiana, Illinois, Michigan, Wisconsin, Missouri, and Nebraska.

12. *Hyperaspis fimbriolata* Melsheimer. — The typical form of *fimbriolata* Melsh., is the most common species of the *fimbriolata* group found in the Upper Mississippi Basin. Specimens have been seen from Indiana, Illinois, Michigan, Wisconsin, Iowa, and Missouri.

13. *Hyperaspis dissoluta coloradana* Casey. — A doubtful subspecies of *dissoluta* Crotch, so designated by Dobzhansky (1941). Dobzhansky listed it from Cook Co., Minn. It has not been seen during the course of the present study.

14. *Hyperaspis disconotata* Mulsant. — Easily distinguished from the other members of the genus by the humeral spot. Recorded from Indiana, Illinois, Minnesota, and Missouri. Several specimens having the equilaterally triangular humeral spots of the subspecies *Hyperaspis disconotata troglodytes* Mulsant have been seen from Missouri and Iowa.

15. *Hyperaspis undulata* (Say). — The most common species of the genus in the midwest. Recorded from all states of the Upper Mississippi Basin.

16. *Hyperaspis octavia* Casey. — Resembles *undulata* (Say) but the lateral vittae are broken into the component spots. Specimens from Michigan and Minnesota have been seen by the author.

17. *Hyperaspis punctata* Leconte. — A single specimen from St. Louis, Mo., XII-6-03, G. W. Bock, is in the Bock collection at the University of Missouri. This seems to be a western species and may be collected rarely in Missouri and in the states immediately to the west.

18. *Hyperaspis annexa* Leconte. — Dobzhansky (1941) records this species from Idaho and California only. Two specimens from the midwest have been seen by the author; Hessville, Indiana, A. B. Wolcott, in the Blanchard collection at the Museum of Comparative Zoology and N. Illinois, June, in the Michigan State College collection.

19. *Hyperaspis quadrivittata* Leconte. — No previous records of this species have been recorded east of Nebraska. Specimens have been seen from Indiana, Illinois, Michigan, Iowa, Kansas, and Nebraska.

20. *Hyperaspis brunnescens* Dobzhansky. — This species is closely related to *quadrivittata* Lec. but can be separated from the latter by the alutaceous interstices of the elytra. It was described from Illinois by Dobzhansky (1941). Additional specimens have been seen as follows: Illinois, state record, Bolter collection, Illinois Natural History Survey; Mineral springs, Ind. XI-15-11, F. Psota, in the Chicago Natural History Museum.

21. *Hyperaspis moerens* (Leconte). — Evidently a very rare species. The only specimen seen was the type from Lake Superior, Mich.

22. *Hyperaspis bolteri* Leconte. — Another rare species. No collections made in recent years have been seen. Dobzhansky (1941) records it from Illinois and Kansas. I have seen several specimens from Illinois as well as specimens from Pine, Ind. in the Fall collection at Harvard, and from Lake Co., Ind. in the Purdue collection.

23. *Hyperaspidius vittigerus* (Leconte). — I include here as synonyms *trimaculata* Cr., *oblongus* Csy., and *wolcotti* Nunen. Nunemacher (1911) undoubtedly described the female of *vittigera* (Lec.) as *wolcotti*. Specimens in the collection of the Chicago Natural History Museum from Hessville, Indiana collected by A. B. Wolcott and labeled 'from the type lot' (i.e. of Wolcott) are all *vittigera* (Lec.). I have seen specimens from Indiana, Illinois, Minnesota, Iowa, and Kansas.

24. *Hyperaspidius transfugatus* Casey. — A very rare species not commonly found in collections. The species was described from Massachusetts in 1899. A series of specimens from Jackson Co., Minn., 1896 and Spirit Lake, Iowa, 1896 are in the G. W. Marshall collection at the University of Wisconsin. I have seen a single specimen from New Jersey in the collection of the Ohio State University.

25. *Hyperaspidius militaris* (Leconte). — A southern species, seldom encountered in the Upper Mississippi Basin. There is a single specimen from Spirit Lake, Iowa, collected by Liebeck, in the Fall collection at Harvard, and a specimen from Douglas Co., Kan. in the Snow collection at the University of Kansas.

26. *Brachyacantha dentipes* (Fab.). — The forms *socialis* Csy., *separata* Leng, and *tau* Leconte have been included here as synonyms. In the latter the pale areas of the pronotum and elytra are greatly developed and confluent so as to leave the body yellow with black markings. This phenomenon is common in several other species of this genus. Recorded from Ohio, Illinois, Iowa, Missouri, Kansas, and Nebraska.

27. *Brachyacantha ursina* (Fab.). — The most common species of the genus in North America. The form *albifrons* Say is included as a synonym as the male genitalia are identical and all of the intergrades between the typical *ursina* (Fab.) and *albifrons* Say have been seen from the middle states. The darker forms predominate in all cases.

28. *Brachyacantha felina* (Fab.). — This species is the smallest of the North American species of the genus. It is liable to confusion with *bolli* Crotch but is more elongate and the elytral spots are smaller. Recorded from Ohio, Indiana, Illinois, Michigan, Wisconsin, Iowa, Missouri, Kansas, and Nebraska.

29. *Brachyacantha bolli* Crotch. — This species is more common in the southern states of the region studied. Recorded from Ohio, Illinois, Michigan, Minnesota, Iowa, Missouri, and Kansas.

30. *Brachyacantha quadripunctata* Melsh. — Blatchley (1910) lists this species abundant at times on aphid-infecsted maples in Indiana. Recorded from Missouri and Kansas. *Basalis* Melsh., *confusa* Muls., and *flavifrons* Muls. are included as synonyms, as there is little evidence to recommend sub-specific rank.

31. *Microweisea misella* (Leconte). — This very small species is common throughout Illinois, Indiana, Michigan, Iowa, Missouri, and Kansas.

32. *Microweisea marginata* (Leconte). — I have seen the type from Marquette, Mich., a state record (Mich.) in the Horn collection, a specimen from Detroit, Mich. in the Michigan State College collection, and a specimen from Marquette, Mich. in the Casey collection at Washington, D. C. The species is quite rare and no recent collections have been seen.

33. *Stethorus punctum* (Leconte). — Orchards heavily infested with the two spotted spider mite invariably contain large numbers of this species which occurs throughout the region studied.

34. *Scymnus* (*Scymnus*) *americanus* Muls. — Easily recognized by the large size and the incomplete metacoxal arcs. The elytra occasionally may be entirely black as in the case of the single specimen named *rusticus* by Col. Casey and later *indianensis* by Weise. The two names become synonyms. The species has been collected in all states of the region, except Wisconsin where it undoubtedly occurs.

35. *Scymnus* (*Scymnus*) *circumspectus* Horn. — This is a southern species which has been collected only once in the Upper Mississippi Basin: Grassy, Mo., V-30-43, R. C. Froeschner.

The subgenus *Pullus* Mulsant has been in considerable confusion for the past several years. The following revision of the midwestern species is based on the secondary sexual characters of the first and fifth ventral segments of the male abdomen as well as the primary sexual characters of the male. The terminology of the sexual characters used in the foregoing key and the following descriptions is that of Verhoeff (1895) followed by Dobzhansky (1931) and Chapin (1946).

36. *Scymnus* (*Pullus*) *punctatus* Melsheimer (Figs. 2 and 14) 1847 *Scymnus punctatus* Melsheimer, Proc. Acad. Nat. Sci. Phila. 3:180.

Elongate-oval, slightly convex, shining; head and pronotum black, punctulation fine; elytra black with a median red spot, punctulation deeper and more distinct than that of pronotum; abdomen black; fifth ventral abdominal segment of males faintly impressed at middle of posterior margin; first ventral segment not modified at middle; prosternum with incomplete carinae from the base to about one-half to two-thirds the length; femora and tibiae black or dark brown, tarsi and mouthparts pale; male genitalia (Fig. 14) without ventral alae; penis broadly spade-shaped, rounded laterally; parameres a little longer than penis, ornamented with long hairs externally for over two-thirds the length, internally for only one-fourth the length; siphonal capsule (Fig. 2) angulately truncate at the proximal end. Length, 1.5–1.8 mm.; width, 1.0–1.2 mm.

Of the specimens seen during the course of the study, all showed incomplete

prosternal carinae, contrary to Horn (1895), and no signs of the modified first ventral abdominal segment in the males as noted by Casey (1899). The red spot of the elytra is quite variable in size and shape.

Distribution records:

OHIO: Hocking Co., VIII-30, D. J. and J. N. Knull (O.S.U.).

ILLINOIS: State record, Bolter Coll.; Galesburg, VII-16-92, Stromberg Coll. (I.N.H.S.): Dekalb Co., F. H. Webster (U. of Kan.): St. Clair Co., IV-26-02, G. W. Bock (U. of Mo.).

WISCONSIN: Delaven, VI-30-40, H. Dybas (C.N.H.M.).

MINNESOTA: Olmsted Co., C. N. Ainslie; St. Anthony Park (St. Paul), Lugger Coll. (U. of Minn.).

IOWA: Ames, V-18-36, H. Gunderson; Traer, VII-1-31, G. C. Decker and H. M. Harris (I.S.C.).

MISSOURI: Falling Springs (St. Clair Co.), IV-20-01, G. W. Bock (U. of Mo.).

KANSAS: Douglas Co., F. H. Snow (U. of Kan.): Onaga, VI-24-09, Bock Coll. (U. of Mo.)

The author has collected this species, in company with *Stethorus punctum* (Lec.), on cedar near Ames, Iowa. The two species were presumably feeding on the two-spotted spider mite, which was present on the tree in small numbers.

37. *Scymnus* (*Pullus*) *nanus* Leconte (Figs. 1 and 5) 1852 *Scymnus nanus* Leconte, Proc. Acad. Nat. Sci. Phila. 6:141.

Oval, convex; head black or dark brown, paler toward the clypeus; pronotum entirely black or with nebulously pale apical angles; elytra black, apex finely pale, more coarsely punctulate than pronotum; body beneath black, legs red or pale brown; fifth ventral abdominal segment of male broadly, not deeply emarginate or impressed; prosternal carinae variable, incomplete at about one-half the length or complete to the anterior margin; male genitalia (Pl I, Fig. 5) with penis narrowing rapidly from a broad base to a sharp point, sides not broadly curved as in *punctatus* Melsh.; penis shorter by nearly one-half than the parameres which are ornamented with long hairs on the external margin from the middle of the length to near the apex; apex of parameres with a tuft of long hairs; siphonal capsule (Fig. 1) with a rounded knob. Length, 1.5–1.8 mm.; width, 1.0–1.5 mm.

Horn (1895) reported *nanus* Lec. lacking elevated lines (carinae) on the prosternum. An examination of Leconte's type of *nanus* revealed prosternal carinae present. The carinae of the type join in a very broad arc at about the middle of the length. Casey (1899) did not clearly indicate the presence or absence of prosternal carinae in this species.

Distribution records:

ILLINOIS: Quincy, VI-11, 30-88, Hart. Coll. (I.N.H.S.).

MICHIGAN: State record, Frederick Allen in Eddy Coll. (M.C.Z.): Cheboygan Co., VII-30-35, M. Sanderson (U. of Kan.).

MINNESOTA: Hennepin Co. (Lake Calhoun), VI-12-21, W. E. Hoffman (U. of Minn.).

IOWA: State record (I.N.H.S.).

NEBRASKA: (—) Leconte's type may have come from Nebraska. When published the locality was given as "Missouri Territory."

This is a very scarce species and is seldom collected. Large series containing both sexes are not available. The males may be identified with certainty by the sexual characters.

38. *Scymnus* (*Pullus*) *kansanus* Casey (Figs. 3 and 13) 1899 *Scymnus* (*Pullus*) *kansanus* Casey, Jour. N. Y. Ent. Soc. 7:142.

Elongate-oval, narrowed behind, convex; head yellow or red; pronotum yellow or red, often with an irregular black spot or nebulous dark area in front of scutellum; elytra longer than wide, black, finely red at apex, abdomen black or dark brown, widely pale at apex; fifth ventral abdominal segment of males with a faint median impression; first ventral abdominal segment of males not modified, uniformly punctulate and pubescent; prosternal carinae greatly variable joining at about the middle of the length in a wide arc or complete to the anterior margin; male genitalia (Fig. 13) with penis spade-shaped but more elongate than in *punctatus* Melsh. and *nanus* Lec.; inner margins of penis sinuate; ventral alae lacking; parameres extending well beyond the tip of the penis, with widely spaced short hairs

along the length of the external margins and a dense tuft of long hairs at the apex; siphonal capsule (Fig. 3) hatchet-shaped. Length, 1.8–2.2 mm.; width, 1.3-1.5 mm.

Casey (1899) described this species from a single male from Kansas. No mention was made in the description of the shallow impression of the fifth ventral abdominal segment of the male. Casey separated *kansanus and cervicalis* Muls. from closely related species by the entirely pale pronotum. This character is worthless, or at most of little value, as long series of *kansanus* Csy. invariably contain individuals in which the pronotum is marked with dark color just in front of the scutellum. The species *kansanus* Csy. is evidently not closely related to *cervicalis* Muls., as indicated by Casey, but is allied to *nanus* Lec. and *punctatus* Melsh. as indicated by the male genital characters and the variable prosternal carinae. Horn (1895) confused *kansanus* Csy. and *cervicalis* Muls. An examination of the Horn material labeled *cervicalis* Muls. revealed the Missouri specimens to be *kansanus* Csy. Wilson (1927) has clearly mistaken *kansanus* Csy. for *cervicalis* Muls. as evidenced by the siphonal capsule of the genitalia figured.

Distribution records:

OHIO: Hocking Co., VIII-1, D. J. and J. N. Knull; Columbus (O.S.U.); Logan Co., VIII-8-32, D. Murray (U. of Minn.).

INDIANA: Starke, Kosciusko, Vermillion, Marshall, Posey, and Knox counties, W. S. Blatchley (Purdue): E. Gary, V-11-13 (U. of Mich.): Lafayette, VIII-5-05, A. B. Wolcott (C.N.H.M.).

ILLINOIS: Glen View, VI-22-18, E Liljeblad; Riverside, VI-21-15; Willow Springs, VI-21-15 (U. of Mich.) Kappa, Aug. 1898; New Milford, VII-2-36, Ross and Burks; Putman Co., VI-5-32, N. O. Glenn; White Heath, V-29-38, J. C. Dirks; Putnam, XI-2-33 (I.N.H.S.) Willow Springs, VI-29-13, F. Psota (C.N.H.M.).

MICHIGAN: State records, Blanchard Coll. (M.C.Z.). Chope Coll. (C.N.H.M.), and (M.S.C.).

MINNESOTA: Mille Lacs. VI-2-37, R. Handford; S. E. tip Houston Co., V-24-36, H. R. Dodge (U. of Minn.).

WISCONSIN: Dalaven, VII-23-4, H. Dybas (C.N.H.M.).

IOWA: State record (M.S.C.): Ft. Madison (Purdue): Warren Co., VIII-12-33, Barker; Louisa Co., VI-9-38, B. Berger (I.I.S.).

MISSOURI: Atchison (CWW), Boone (CWW), Buchanan (CWW), Cape Gir. (CWW), Clay (RCF), Macon (RCF), Monroe (CWW), Nodaway (RCF), Pike (WSC), Schuyler (RCF), Scotland (RCF), and St. Louis (CWB) counties.

KANSAS: Douglas Co., F. H. Snow; Gove Co., J. H. Snow (U. of Kan.).

NEBRASKA: 30 mi. S. Valentine. VI-9-50. Slater, Hicks, and Laffoon (I.S.C.).

The species is predominately northern as indicated by the Missouri records. Of these, the Cape Girardeau Co. record is the only locality south of the Missouri River where *kansanus* Csy. occurred

39. *Scymnus* (*Pullus*) *cervicalis* Mulsant (Fig. 19) 1850 *Scymnus* (*Pullos*) *cervicalis* Mulsant, Spec. Trim Sécuripalp., p. 984.

Oval, rounded, convex; head and thorax yellow or reddish yellow; pronotum markedly sinuate each side of scutellum; elytra black, narrowly bordered with reddish yellow at apex; abdomen black except fourth and fifth segments reddish yellow; fifth ventral abdominal segment of males with a deep rounded fovea extending into the sixth segment; first abdominal segment of males unmodified; prosternal carinae prominent, slightly convergent throughout the length which reaches the anterior margin; male genitalia (Fig. 19) with penis longer than parameres, narrowed sharply past the middle, apex slender and pointed; ventral alae thick and club-like, heavily sclerotized, shorter than penis; parameres much shorter than penis, ornamented with a thick tuft of hairs originating on the side well before the apex; hairs of parameres reaching as far as the apex of the penis. Length, 1.8–2.0 mm.; width, 1.1–1.4 mm.

As noted in the remarks on the preceding species, *cervicalis* Muls. is often mistaken for *kansanus* Csy. in collections. It may be separated from *kansanus* by the more rounded and convex form, the pronotum being completely yellow or pale, by the deep and rounded fovea of the fifth ventral segment of the male and by the complete prosternal carinae. The male genitalia of the two species are quite unlike.

Horn (1895) thought the prosternal carinae of *cervicalis* Muls. to be variable in extent, but this has not been found to be true by the author. Horn, as noted under *kansanus* Csy., was working with a mixed series of *kansanus* and *cervicalis*.

Distribution records:

OHIO: Vinton, XII-6-00; Scioto, VI-17-44, D. J. and J. N. Knull (O.S.U.).

INDIANA: Marshall Coll. VI-13-03, W. S. Blatchley; Starke Coll. VI-19-03, W. S. Blatchley; Harrison Co., VI-20-34, D. W. LaHue (Purdue).

ILLINOIS: State record, Liebeck Coll. (M.C.Z.): Dubois, V-24-17 and VIII-8-17; Ashley, VIII-7-17; Herod, VI-23-27, T. F. and R. G. (I.N.H.S.).

MICHIGAN: Detroit (U. of Kan.): State Record, E. Chope Coll. (C.N.H.M.).

MISSOURI: Barry (RCF). Bollinger (CWW), Boone (RCF), Butler (EHF), Cape Gir. (CWW), Carter (RCF), Cole (RCF), Dallas (CWW), Douglas (RCF), Franklin (RCF), Iron (RCF), Lawrence (CWW), McDonald (RCF), Monroe (CWW), Phelps (RCF), Pike (WSC), Pulaski (RCF), Saline (RCF), Shannon (CWW), Stoddard (RCF), St. Francis (RCF), St. Louis (RCF), and Washington (RCF) counties.

KANSAS: Douglas Co., F. H. Snow (U. of Kan.).

The species is well distributed in the southern part of the western section of the Upper Mississippi Basin. It is common south of the Missouri River and scarce north of that latitude. No Iowa, Nebraska, or Minnesota specimens have been seen. Blatchley (1910) records *cervicalis* Muls. as frequent throughout Indiana, but many of the specimens labeled *cervicalis* Muls. by Blatchley were *kansanus* Csy., which generally occurs further north.

40. *Scymnus* (*Pullus*) *Lodi* Stehr 1946 *Scymnus lodi* Stehr, Ann. Ent. Soc. Amer. 39:80.

The following is extracted from the original description by Stehr (1946, p. 80): . . . metacoxal line forming a complete arc . . . body is very broadly oval and convex . . . entire upper surface is rufo-testaceous except for a parabolic black spot on the middle of the base of the prothorax extending to within one-fourth of the apex. The under surface is testaceous except that the meso and metepimera are fuscous and there is a fuscous tinge along the outer margin of the epipleurae . . . The last ventral segment of the male is evenly arcuately emarginate at the middle. Length 2.6 mm., width 2.1 mm.

Distribution records:

OHIO: Known only from the holotype, a male in Prof. Stehr's collection; Lodi Township, Athens Co., Ohio, Sept. 27, 1945, collector Wm. C. Stehr.

This species bears some little resemblance to the pale forms of *brullei* Mulsant. Professor Stehr (1949) says the first ventral segment of *lodi* is unmodified, the fifth is less deeply foveate than in *brullei* Muls. and the male genitalia are, "not much more than half as long as those of *brullei* Muls. and are of much stouter build." These differences as noted will serve to separate the two species.

41. *Scymnus* (*Pullus*) *cinctus* Leconte (Fig. 9) 1852 *Scymnus cinctus* Leconte, Proc. Acad. Nat. Sci. Phila. 6:137.

Oval, convex; head reddish yellow; pronotum reddish yellow with a parabolic black spot from the base reaching nearly to the anterior margin; elytra reddish yellow with a large triangular common black area extending down the suture from the base, a little wider at the base than the black spot of the pronotum; body beneath black; first ventral abdominal segment of males with an oval depressed area at the middle with the pubescence shorter than that immediately surrounding it; fifth ventral abdominal segment of males very slightly sinuate along the posterior margin; abdominal segment; prosternal carinae convergent, complete to the anterior margin; male genitalia (Fig. 9) with penis longer than parameres, tapering to an acute point from the posterior third to the apex, widest at the base; parameres slender at base, tipped with long tufts of hairs. Length, 2.4 mm.; width, 1.7 mm.

Distribution records:

IOWA: Shenandoah, VI-6-50, VI-4-50, W. S. Craig.

MISSOURI: State record, Nov. 1947, hibernating in bunch grass; Hayti, VIII-11-39, R. C. Froeschner; Tyler, X-7-39, R. C. Froeschner; Mound City, VI-8-50, R. C. Froeschner.

KANSAS: Sterling, VI-25-36, D. R. Lindsay; Finney Co., VIII-13-24, Beamer and Lawson (U. of Kan.).

From the existing distribution records *cinctus* Lec. is a western or southwestern species which is occasionally collected in the above states. The species may be confused with similarly marked pale forms of *brullei* Muls. if the characters of the male abdomen are not plainly visible.

42. *Scymnus* (*Pullus*) *festatus* new species (Fig. 22)

Oval, convex; head reddish orange; pronotum reddish orange with a nebulous black area at the base in front of the scutellum; elytra black, broadly pale at apex; apical pale area covering about one-fifth of the length, advancing a little further along the suture than lateral margin; abdomen orange or reddish orange, finely black at base; first ventral abdominal segment of males not modified; fifth ventral abdoninal segment of males broadly sinuate at posterior margin, forming a deep fovea with the impressed sixth segment; mesosternum and metasternum black; prosternum reddish orange, finely black at base; prosternal carinae nearly parallel for three-fourths the length, slightly convergent thereafter and joining in a broadly rounded curve at the anterior margin; male genitalia (Fig. 22) without ventral alae; penis longer than parameres, widest at middle, broadly curved from the base, bulbous, narrowed at two-thirds the length to form a subparallel-sided distal portion terminating in an angular point; internal margins of penis with angulate processes; dorsal surface of penis with a sharp process at the base of the subparallal portion; parameres curved ventrad with the bulbous portion of the penis, tufted with long hairs rising from the dorsal margin just before the apex. Length, 2.3 mm.; width, 1.7 mm.

Holotype (male) and one *Paratype* (male). Hocking Co., Ohio, V-8-38, D. J. and J. N. Knull, in the collection of Ohio State University.

This species resembles *semiruber* Horn somewhat in appearance but is much larger and more robust. The male genitalia differ from allied species especially in the elongate apex of the penis.

43. *Scymnus* (*Pullus*) fraternus Leconte (Fig. 11)

1852 *Scymnus fraternus* Leconte, Proc. Acad. Nat. Sci. Phila. 4:138.
1852 *Scymnus haemorrhous* Leconte, Proc. Acad. Nat. Sci. Phila. 4:138.
1874 *Scymnus fraternus* Crotch, Rev. Cocc., p. 264.
1895 *Scymnus fraternus* Horn, Trans. Amer. Ent. Soc. 22:101.
1899 *Scymnus* (*Pullus*) *creperus* var. *fraternus* Casey, Jour. N. Y. Ent. Soc. 7:140.
1901 *Scymnus dentipes* Fall, Occ. Papers Calif. Acad. Sci. 8:234.
1904 *Scymnus dentipes* Fall, Ent. News 18:176.
1910 *Scymnus fraternus* Blatchley, Indiana Dept. Geol. Bull. 1, p. 529.
1920 *Scymnus creperus* var. *fraternus* Leng, Cat. Col. N. Am., p. 213.
1920 *Scymnus haemorrhous* Leng, Cat. Col. N. Am., p. 213.
1927 *Scymnus haemorrhous* Wilson, Psyche 32:170.
1930 *Scymnus fraternus* Stehr, Minn. Agr. Exp. Sta. Tech. Bull. 75, p. 49.
1930 *Scymnus haemorrhous* Stehr, Minn. Agr. Exp. Sta. Tech. Bull. 75, p. 50.
1931 *Scymnus fraternus* (= *creperus*) Korschefsky, Col. Cat. pars 118, p. 157.
1931 *Scymnus haemorrhous* Korschefsky, Col. Cat. pars 118. p. 159.

Broadly oval, rounded, convex; head yellowish red; pronotum black with wide lateral margins of yellowish red, or yellowish red with a median parabolic black spot extending from less than one-half the length from the base to the full length of the pronotum; elytra black, shining, apex transversely yellow for one-sixth the length; pale apical area of elytra extending further along the margin than suture; first and second ventral abdominal segments black, remaining segments yellowish red; first ventral abdominal segment of males not modified at middle; fifth ventral abdominal segment of males broadly sinuate, impressed with the sixth to form a shallow fovea; prosternum yellowish red, narrowly black at base; prosternal carinae convergent, complete to the anterior margin; male genitalia (Fig. 11) with penis asymmetrical, inner margin with a sharply pointed process on one side; parameres shorter than penis. Length, 2.9–2.5 mm.; width, 1.6–1.8 mm.

Authors have long considered the species *fraternus* Lec. a variety of *creperus* Muls. In the original description of *creperus* Muls. the apical pale margin of the elytra is described as red-brown covering one-fifteenth of the length of the elytra. The specimen or specimens before Mulsant, at the time the original description was written, came from the vicinity of New Orleans, La. There are perhaps a dozen species of *Scymnus* (*Pullus*), occurring in the vicinity of New Orleans, having the

elytra narrowly bordered with pale brown or red. The apical pale area of *fraternus* Lec. covers much more than one-fifteenth of the length and is usually constant in extent. The male genitalia of *fraternus* Lec., as noted above, are unique in that the penis is asymmetrical and not liable to confusion with other known species of *Pullus,* in which the penis is bilaterally symmetrical.

A study of Leconte's types of *fraternus* and *haemorrhous* revealed that the type of the latter is a female *fraternus* Lec. having the black area of the pronotum much expanded. Fall (1907) placed *dentipes* Fall as a synonym of *haemorrhous* Lec.

Distribution records:

OHIO: Columbus and Vinton (O.S.U.).

INDIANA: Crawford Co., V-24-03, W. S. Blatchley (Purdue).

ILLINOIS: Glen Carbon, VIII-22-42, J. Slater (I.S.C.): Bowmanville, Chicago, and Rock Island (U. of Mich.): Savanna, VII-19-32, Forbes *et al.*; Alto Pass, V-7-17: Fountain Bluff, V-15-32, Frison and Ross: Putnam Co., VII-17-32, M. O. Glenn: Metropolis, VIII-19-91, Shiga (I.N.H.S.): Glencoe, V-31-09, Gerhard (C.N.H.M.).

MICHIGAN: Detroit (M.S.C.): Cheboygan Co., VII-17-36, M. Sanderson (U. of Kan.).

WISCONSIN: Delaven, VII-23-40, H. Dybas (C.N.H.M.): Madison, VIII-20-17, C. L. Fluke (U. of Wis. Ent.): Eagleton, VIII-29-37, L. R. Penner (U. of Minn.).

MINNESOTA: Plummer, VI-6-33, D. Denning; Chicago Co., VII-15-11; Frontenac, V-29-30, Wm. C. Stehr; Olmsted Co., C. N. Ainslie; Mille Lacs Co., VI-2-37, C. E. Mickel; Fillmore Co., V-24-38, P. M. Schroeder; White Bear (Manitou Is.), VII-3-21, W. E. Hoffman (U. of Minn.).

IOWA: Sioux City, VI-24-35, C. N. Ainslie (U. of Minn.): Tama Co., VIII-8-33, Jaques; Monona Co., VII-1-32, Russell; Dickinson Co., VIII-20-33, Barker; Clayton Co., VIII-2-32, Cronn; Bremer Co., VII-11-33, H. E. J.: Muscatine, VII-10-36, B. Berger (I.I.S.).

MISSOURI: Barry (RCF), Bollinger (RCF), Boone (WSC), Cape Gir. (CWW), Carter (RCF), Dunklin (RCF), Jefferson (RCF), McDonald (RCF), Perry (CWW), Shannon (CWW), St. Francis (EHF), Taney (CWW), and Wayne (RCF) counties.

44. *Scymnus* (*Pullus*) *rubricaudus* Casey (Fig. 20)

1899 *Scymnus* (*Pullus*) *rubricauda* Casey, Jour. N. Y. Ent. Soc. 7:141.
1899 *Scymnus* (*Pullus*) *texanus* Casey, Jour. N.Y. Ent. Soc. 7:141.
1899 *Scymnus* (*Pullus*) *chromopyga* Casey, Jour. N.Y. Ent. Soc. 7:141.
1910 *Scymnus rubricauda* Blatchley, Indiana Dept. Geol. Bull. 1, p. 530.
1920 *Scymnus texanus* (*fraternus* Lec.) Leng, Cat. Col. N. Am., p. 213.
1920 *Scymnus rubricauda* Leng, Cat. Col. N. Am., p. 213.
1920 *Scymnus chromopyga* Leng. Cat. Col. N. Am., p. 213.
1931 *Scymnus* (*Pullus*) *rubricauda* Korschefsky, Col. Cat. pars 118, p. 165.
1931 *Scymnus texanus* (*creperus* Muls.) Korschefsky, Col. Cat. pars 118, p. 157.
1931 *Scymnus* (*Pullus*) *chromopyga* Korschefsky, Col. Cat. pars 118, p. 156.

Elongate-oval, little convex; head reddish yellow; pronotum black with the anterior angles reddish yellow, or the pale area of the anterior angles is so expanded as to leave the pronotum reddish yellow with a large median parabolic black spot at the base; elytra black with large reddish yellow apical areas extending in some specimens as far as the apical third; anterior margins of pale area of the elytra arcuate, leaving a sharp point of black color extended down the suture; abdomen black at base, pale at apex, first ventral abdominal segment of males not modified at middle; fifth ventral abdominal segment of males with a distinct fovea at posterior margin; prosternal carinae convergent, complete to the anterior margin; genitalia of males (Fig. 20) with penis longer than parameres, lobed internally, sharply pointed at apex; ventral alae lacking; parameres rising in a half spiral from basal plates, tufted with hairs at apex. Length, 1.8–2.0 mm.; width, 1.1–1.4 mm.

An examination of Casey's types of *texanus, rubricauda,* and *chromopyga* revealed no differences between the three. Although *rubricauda* is preceded by *texanus* on the page, the name *rubricaudus* is retained since the description fits a larger number of cabinet specimens and the descriptive name is preferable.

Distribution records:

OHIO: Fairfield, Delaware, and Greene counties; Athens and Clifton (O.S.U.).

INDIANA: Posey Co., VI-18-23, S. W. B. (A.M.N.H.): Hessville, Liljeblad; Putnam Co., Marion Co., W. S. Blatchley (U. of Mich.): Perry, Lawrence, Vigo, Floyd, Marion, Knox, Clark, and Starke counties, W. S. Blatchley (Purdue): Knox Co., VII-14-04, A. B. Wolcott (C.N.H.M.).

ILLINOIS: State record (N. Ill.), W. G. Dietz (M.C.Z.) Urbana, V-4-88, Hart Coll.; Castle Rock, XII-9-32, Frison and Ross (I.N.H.S.): River Forest, VI-27-04, A. B. Wolcott (C.N.H.M.).

MICHIGAN: Detroit (M.S.C.).

WISCONSIN: Delaven, VII–5–40, H. Dybas (C.N.H.M.).

MINNESOTA: Houston Co., V-24-37, C. E. Mickel; Mille Lacs, VI-2-37, R. H. Daggy; St. Anthony Park, VI-3-21, W. E. Hoffman; Chicago Co., VII-15-9, C. N. Ainslie: Olmsted Co., 1897, C. N. Ainslie; Frontenac, V-29-30, Wm. C. Stehr (U. of Minn.).

IOWA: Sioux City, VI-3-31, C. N. Ainslie (U. of Minn.): Van Buren Co., V-13-32, Barker; Henry Co., V-9-35, Carlson; Appanoose Co., V-12-50, Wettach (I.I.S.); Ames, V-15-50, J. A. Slater; Ledges St. Park, V-31-50, J. Laffoon (I.S.C.): Hamburg, VIII-3-50, W. S. Craig.

MISSOURI: Atchison (RCF), Barry (RCF), Bollinger (RCF), Boone (RIW), Cape Gir. (CWW), Clark (RCF), Dunklin (RCF), Jefferson (RCF), Monroe (CWW), New Madrid (WSC), Perry (CWW), Schuyler (RCF), St. Charles (CWW), St. Francis (RCF), St. Louis (RCF), and Washington (CWW), counties.

KANSAS: State record (types) Casey Coll. (N.M.C.): Coffeyville VII-11-39, L. W. Hapner (U. of Kan.).

NEBRASKA: Ashland (U. of Neb.): Valentine, VI-8-50, Hicks, Slater, Laffoon (I.S.C.).

45. *Scymnus* (*Pullus*) *brullei* Mulsant (Fig. 25)

1850 *Scymnus* (*Pullus*) *brullei* Mulsant, Spec. Trim. Sécuripalp., p. 984.
1895 *Scymnus brullei* Horn, Trans. Amer. Ent. Soc. 22:101.
1899 *Scymnus* (*Pullus*) *haemorrhous* Casey, Jour. N. Y. Ent. Soc. 7:140.
1899 *Scymnus* (*Pullus*) *haemorrhous* var. *divisus* Casey, Jour. N. Y. Ent. Soc. 7:140.
1899 *Scymnus* (*Pullus*) *haemorrhous* var. *laurenticus* Casey, Jour. N. Y. Ent. Soc. 7:140.
1899 *Scymnus* (*Pullus*) *haemorrhous* var. *subaenus* Casey, Jour. N. Y. Ent. Soc. 7:140.
1920 *Scymnus brullei* Leng, Cat. Col. N. Am., p. 213.
1931 *Scymnus brullei* Korschefsky, Col. Cat. pars 118, p. 155.

Oval, convex; head yellow, brown, or yellow with the base black; pronotum black with yellow lateral margins wide or narrow, inner margins arcuate; elytra with large arcuate apical pale areas covering from one-fourth to the entire length of the elytra; the elytra may appear entirely yellowish red or pale brown with a narrow black nebulous vitta along the lateral margins from the humeral angles for about one-half the length; abdomen completely black or with the first two segments black, remaining segments yellowish red; first ventral abdominal segment of males with distinct patches of densely-set and long pubescence on each side of the midline; fifth ventral abdominal segment of males deeply sinuate in a wide arc; prosternal carinae convergent, complete to the front margin; legs yellowish red; male genitalia (Fig. 25) with penis curved slightly ventrad at the distal end; ventral alae bilobed dorso-ventrally, dorsal lobes longer; parameres shorter than penis or ventral alae, ornamented dorsally with a row of hairs and apically with tufts of hairs; siphonal capsule convex above the point of attachment to the trabes; siphonal tube reduced in size from middle to the distal end. Length, 2.2–2.4 mm.; width, 1.5–1.7 mm.

Distribution records:

OHIO: State record, Leconte Coll. (M.C.Z.): Athens, V-22-32, Wm. C. Stehr; Greene Co., V–12, D. J. and J. N. Knull; Delaware Co., D. J. and J. N. Knull; Ashtabula, VII-21-99 (O.S.U.).

INDIANA: Fountain, Knox, Posey, Putnam, Lawrence, Vermillion, Tippecanoe, Lafayette, Warren, Clark, Marion, and Tipton counties, W. S. Blatchley (Purdue): Lafayette and E. Gary (U. of Mich.): Pine, VII-14-04, A. B. Wolcott (C.N.H.M.).

ILLINOIS: White Heath, V-11-40 (I.S.C.): Brook, Fall Coll.; Galesburg, Liebeck Coll., (M.C.Z.): Bowmanville, Willow Springs, Riverside, Rock Island, and Edgebrook (U. of Mich.): Hardin, Algonquin, Muncie, Carlinville, Urbana, Mahomet, Oakwood, and Shawneetown (I.N.H.S.): Willow Springs, VI-13-09, F. Psota; Starved Rock, V-9-37, H. Dybas; River Forest, VI-5-04, A. B. Wolcott (C.N.H.M.).

MICHIGAN: State record, Chope Coll. (C.N.H.M.).

WISCONSIN: Wittenberg, VIII-27-37, L. R. Penner (U. of Minn.).

MINNESOTA: Chicago, Houston, Norman, Washington, Hennepin, Otter, Otter Tail, and Olmsted counties; Frontenac, Plummer, Crookston, Pine City, and Eagle Bend (U. of Minn.).

IOWA: Ames, common April-October; Boone, Shenandoah, Camp Dodge, Maquatah Caves St. Park, Ledges St. Park (I.S.C.): Keokuk, Lee, Clayton, Fremont, Jefferson, Van Buren, Linn, Washington, and Henry counties (I.I.S.): McGregor (U. of Mich.): Sioux City (U. of Minn.).

MISSOURI: Atchison (RCF), Barry (RCF), Boone (RCF), Cape Gir. (CWW), Howard (WRE), Jefferson (RCF), Monroe (CWW), Newton (RCF), Perry (CWW), Pike (WSC), Saline (RCF), Schuyler (RCF), St. Genevieve (RCF), St. Louis (RCF), and Stone (RCF) counties: Willard, V-1918, Psota Coll. (C.N.H.M.).

KANSAS: State record T. B. A., Blatchley Coll., (Purdue): Onaga and Creve Coeur; Douglas Co.; Lawrence (U. of Kan.).

NEBRASKA: Ashland (M.S.C.): Lincoln, Pine Ridge, Hat Creek, Bellevue, West Point, Ashland, Sioux Co., and Monroe Co. (U. of Neb.).

This is an exceedingly variable species in the area studied. In the eastern states of the Upper Mississippi Basin the pronotal and elytral markings described by Mulsant (1850) predominate. In the western states of the region, particularly Iowa, Nebraska, and Kansas, pale forms in which the black areas of the elytra and pronotum almost disappear, occur frequently in series of the typical dark forms. Only the characters of the first and fifth abdominal segments of the males are constant and serve to identify these forms as *brullei* Muls. In the W. S. Blatchley collection *brullei* Muls. appeared under the labels of *creperus* var. *fraternus* Lec. and *haemorrhous* Lec. The species is widely distributed in the United States from Canada to Florida and Texas. In Iowa *brullei* Muls. is found in large numbers on the early foliage of *Ribes* for two or three weeks in the early spring. Later in the season it is less plentiful, but is taken frequently while sweeping grass and herbs.

46. *Scymnus* (*Pullus*) *marginicollis* Mannerheim (Fig. 10)

1843 *Scymnus marginicollis* Mannerheim, Bul. Soc. Nat. Moscou 16:313.

Oval, rounded, convex; head black, brown, or reddish brown; pronotum orange-brown with a basal black spot variable in size, often expanded to leave the pronotum black with pale anterior angles; elytra black, narrowly tipped with orange-brown at the apex; abdomen black or dark brown; first ventral abdominal segment of males with a prominent tubercle at the middle near the posterior margin; fifth ventral segment of males with a rounded fovea at middle of posterior margin; prosternal carinae distinct, convergent and complete to the anterior margin; prosternum of the same color as pronotum; male genitalia (Fig. 10) with penis broadly rounded at the middle, terminating in a sharp point at apex, longer than parameres; ventral alae lacking; parameres thin, tufted with long hairs at the apex. Length, 2.0 mm.; width, 1.5 mm.

Distribution records:

MICHIGAN: Alger Co., VII-28, 31-1937, H. S. Telford (U. of Minn.).

This is a common species in California, easily recognized by the tubercle of the male abdomen. The male genitalia of the single Michigan specimen are identical with those of California specimens of *marginicollis* Mann. Nothing is known of the circumstances of its capture in Michigan, and it is entirely possible that the species is not indigenous to Michigan. California species of the family occasionally enter eastern states with shipments of citrus fruit.

47. *Scymnus* (*Pullus*) *pulvinatus* new species (Fig. 17)

Oval, slightly elongate, convex; head reddish yellow; pronotum black, sparsely and finely punctulate; elytra black, narrowly bordered with reddish yellow, punctures coarse and closely set; abdomen black, a little paler at the posterior margin;

first ventral abdominal segment of male with an elongate-oval, elevated, and flattened area at the middle, covered by fine pubescence, reaching nearly to the hind margin; posterior margin of elevated area with a small rounded projection; fifth ventral abdominal segment of male sinuate at middle of hind margin, foveate with the sixth segment; sternum black; prosternal carinae convergent, complete to the anterior margin; legs pale brown, femora a little darker than tibiae and tarsi; male genitalia (Fig. 17) with penis longer than parameres, wider at middle than base or apex, bulbous, narrowed gradually at two-thirds and ending in a broad, angulate apex; internal margins of ventral side of penis thin, lightly sclerotized, sinuate, without internal processes; dorsal surface of penis curved gently ventrad to the apex, without a projecting process; parameres shorter than penis, widely separated from penis at base, internal surface concave, curved with the contour of the bulbous penis; apex of parameres broadly flared, ornamented with tufts of hair rising from the dorsal margin. Length, 2.0 mm.; width, 1.5 mm.

Holotype (male). St. Joseph, Mo., VI-7-40, R. I. Wakeman, in the collection of the author.

This specimen was thought to be *marginicollis* Mann. for some time. The appearance of the male first ventral is quite similar at first glance, but closer scrutiny reveals the tubercle of *marginicollis* Mann. is at the posterior margin of the segment, while *pulvinatus* is not as distinctly tuberculate and the projection of the raised area of the segment falls just short of the hind margin. The male genitalia of the two species are quite unlike.

48. *Scymnus* (*Pullus*) *nemorivagus* new species (Figs. 15 and 16)

Oval, rounded, convex; head reddish yellow both sexes; pronotum of male reddish yellow, female with a small, indistinct black spot in front of scutellum; pronotal punctures more distinct and closely set at sides than on disc; elytra black with large reddish yellow apical area; apical pale area of elytra extending further cephalad at the middle of the disc than along suture or margin, reaching a point one-half the length of the elytra; first and second abdominal segments black, narrowly pale at sides, third, fourth, and fifth abdominal segments entirely pale in the male, same in female except base of third broadly black; first ventral abdominal segment of male with a raised median area at the middle, glabrous and slightly impressed; margins of the median glabrous area of male first ventral delimited by long, dense pubescince and closely-set, fine punctures; fifth ventral abdominal segment of male with a rounded fovea at middle extending into the sixth; mesosternum and metasternum black both sexes; prosternum reddish yellow in male, black between the coxae in female; legs reddish yellow both sexes; male genitalia (Figs. 15 and 16) with penis longer than parameres, sides slightly convergent and broadly rounded, ending apically in a short, sharply-pointed process; internal ventral margins of penis with angular projections at base and near middle of length; ventral alae lacking; parameres about one-half as long as penis, curved ventrad along the side of the penis, ornamented with terminal hairs and with a slender tuft of hairs at the middle of the dorsal margin (Fig. 16); sipho markedly narrowed beyond the middle of the length. Length, 2.1–2.2 mm.; width, 1.5–1.6 mm.

Holotype (male) and *Allotype* (female). Hollister, Mo., July 22, 1915, H. H. Knight, in the collection of the author.

This species is closely allied by the genital characters to *rubricaudus* Casey and *marginicollis* Mann. It is easily distinguished from these species by the pale pronotum and large pale apical area of the elytra as well as by the male genitalia. It may be confused occasionally with *festatus* or the pale forms of *brullei* Muls., but can be separated from these species by the characters of the first and fifth ventral segments of the males.

49. *Scymnus* (*Pullus*) *impunctus* new species (Fig. 12)

Elongate-oval, convex; head dark brown basally, yellowish from about the middle to the clypeus in both sexes; pronotum entirely black or black with the anterior angles reddish brown; punctures of pronotum more closely set at sides than middle of disc; elytra black, reflexed head and rarely a narrow adjacent area of the hind margin reddish brown; punctures of elytra distinct, not coarse or deep, pubescence fine, suberect; abdomen black, last two or three segments irregularly pale; first ventral abdominal segment of males with a long spade-shaped glabrous area at the middle; glabrous area of male first ventral extending from the hind margin almost to the anterior margin, delimited in outline by longer pubescence, not delimited by closely set punctures; fifth ventral abdominal segment of males slightly sinuate

and impressed at middle of hind margin; sternum black; prosternal carinae slightly convergent, complete to the front margin; male genitalia (Fig. 12) with penis shorter than ventral alae; penis connected to ventral alae laterally by a heavy membrane; ventral surface of penis with an elevated triangular process from the base, shorter than the dorsal portion of the penis; dorsal portion of penis broadly curved at the sides, forming an oval outline when seen from the ventral aspect; ventral alae broadly curved around the penis, longer and more heavily sclerotized ventrally; parameres almost as long as ventral alae, broad at base, internal margins attached along the dorsal surface of the penis, terminal hair tufts long, extending well beyond the ventral alae and curved toward the midline. Length, 1.7–1.8 mm.; width 1.2–1.4 mm.

Holotype (male). Des Arc., Missouri, V-31-43, R. C. Froeschner.

Allotype (female). Kinsey, Missouri, V-13-50, R. C. Froeschner.

Paratypes (2 males). Gray Summitt, Missouri, V-24-42, R. C. Froeschner; Kinsey, Missouri, V-13-50, R. C. Froeschner.

The types are in the collection of the author. In appearance this species resembles *tenebrosus* Muls. It is, however, generally smaller, more elongate in outline, and the ventral process of the penis is much shorter than in *tenebrosus* Muls. By male genital characters, *impunctus* is allied to *consobrinus* Lec. The type and paratype localities, Iron, St. Genevieve, and Franklin counties, are situated along the eastern border of the Missouri Ozarks.

50. *Scymnus* (*Pullus*) *hortensis* new species (Fig. 26)

Oval, convex, slightly narrowed behind; head reddish yellow, pronotum reddish yellow with an ill-defined black spot in front of scutellum reaching to the middle of the length or a little beyond; punctulation of pronotum distinct and evenly spaced; elytra black, narrowly bordered with reddish yellow for 0.17 mm. at apex; first abdominal segment black, second black with pale margins, third, fourth, and fifth reddish yellow; first ventral abdominal segment of males slightly convex at middle, pubescence dense, directed toward the midline from each side; first ventral of males with a short triangular glabrous area at middle of hind margin, very small and almost lacking in some specimens; fifth ventral abdominal segment of males broadly and deeply sinuate, impressed with the sixth to form a fovea; mesosternum and metasternum black; prosternum reddish yellow, black at base and between the front coxae; prosternal carinae convergent, complete to the anterior margin; legs reddish yellow; male genitalia (Fig. 26) with penis longer than ventral alae and parameres, bluntly rounded at apex; membrane between ventral alae and penis attached well toward end of penis; parameres short, stubby, terminal hair tufts long in proportion to parameres but scarcely reaching the apex of the ventral alae. Length, 1.9–2.3 mm.; width, 1.4–1.7 mm.

Holotype (male). Shenandoah, Iowa, V-24-50, W. S. Craig, in the collection of the author with allotype.

Allotype (female). Shenandoah, Iowa, VI-6-50, W. S. Craig. Collected on sunflower with paratypes of same date.

Paratypes. INDIANA: (Male) Marion Co., X-14-21, W. S. Blatchley: (male and female) Jackson Co., IX-23-38, R. L. Schnell, collected from black walnut and wild cherry; (4 males and 4 females) Daviess Co., forest nursery, VIII-13-38, M. C. Reeves, on persimmon and black locust; (male) Daviess Co., forest nursery, IX-7-38, M. C. Reeves, on Osage orange; all Indiana paratypes in the collection of Purdue University. IOWA: (17 males and 20 females) Shenandoah, VI-6-50, W. S. Craig in the collection of the author; (1 male and 2 females) X-14, 18-39 and V-10-35, C. N. Ainslie, in the collection of the University of Minnesota. MISSOURI: (1 male and 1 female) Cape Gir., VI-4-41 and VI-5-40, C. W. Wingo; (1 male and 1 female) Simcoe, VIII-6-49, C. W. Wingo. All Missouri paratypes are in the collection of the author. KANSAS: (3 males and 5 females) Reno Co., IX-37-19, Wm. E. Hoffman; (1 female) Elk City, V-23-36, M. W. Sanderson. All Kansas paratypes are in the collection of the University of Kansas.

This species is remarkably uniform in the coloration of the upper surface. An occasional specimen will have the basal black spot of the pronotum nearly as long as the pronotum, but in most cases the spot reaches less than half the length of the pronotum. The females are slightly larger than the males, occupying the upper half of the range of dimensions given in the description. The genital characters of the males indicate a close relationship of this species with *puncticollis* Lec.

The paratypes from Springfield, Mo., were taken on aphid-infested sweet peas in company with *Scymnus* (*Diomus*) *terminatus* Say. The allotype and long series

of paratypes from Shenandoah, Iowa, were taken by Mr. Craig on sunflower. A single specimen of *Scymnus* (*Pullus*) *cinctus* Lec. was found in the long series of *hortensis* taken by Mr. Craig.

51. *Scymnus* (*Pullus*) *puncticollis* Leconte (Fig. 21)

1847 *Scymnus collaris* Melsheimer (nec Herbst), Proc. Acad. Nat Sci. Phila. 3:180.
1852 *Scymnus puncticollis* Leconte. Proc. Acad. Nat. Sci. Phila. 6:139.
1874 *Scymnus puncticollis* Crotch, Rev. Cocc., p. 266.
1895 *Scymnus puncticollis* Horn, Trans. Amer. Ent. Soc. 22-102.
1895 *Scymnus collaris* Horn, Trans. Amer. Ent. Soc. 22:103.
1899 *Scymnus* (*Pullus*) *collaris* Casey, Jour. N. Y. Ent. Soc. 7:144.
1899 *Scymnus* (*Pullus*) *indutus* Casey, Jour. N. Y. Ent. Soc. 7:145.
1899 *Scymnus* (*Pullus*) *puncticollis* Casey, Jour. N. Y. Ent. Soc. 7:160.
1910 *Scymnus puncticollis* Blatchley, Indiana Dept. Geol. Bull. 1, p. 530.
1920 *Scymnus collaris* Leng, Cat. Col. N. Am., p. 213.
1920 *Scymnus indutus* Leng, Cat. Col. N. Am., p. 213.
1920 *Scymnus puncticollis* Leng, Cat. Col. N. Am., p. 213.
1927 *Scymnus puncticollis* Wilson, Psyche 32:170.
1929 *Scymnus melsheimeri* Weise, Zool. Jahrb. Suppl. 16:33.
1930 *Scymnus collaris* Stehr, Minn. Agr. Exp. Sta. Tech. Bull. 75, p. 50.
1931 *Scymnus* (*Pullus*) *indutus* Korschefsky, Col. Cat. pars 118, p. 160.
1931 *Scymnus* (*Pullus*) *melsheimeri* Korschefsky, Col. Cat. pars 118, p. 162.
1931 *Scymnus* (*Pullus*) *puncticollis* Korschefsky, Col. Cat. pars 118, p. 165.

Oval, rounded, convex; head brown, pale brown, or reddish yellow with base black; pronotum black, anterior angles or wide lateral and anterior margins reddish yellow; black area of pronotum often reduced to a basal parabolic black spot; elytra black, narrowly bordered with reddish yellow at apex; abdomen black with last two or three segments reddish yellow; first ventral abdominal segment of males with a central, glabrous, triangular or rounded area surrounded by closely-set punctulation and dense pubescence; fifth ventral segment of males with a deeply impressed sinuation at hind margin; prosternal carinae convergent, reaching the anterior margin; legs reddish brown, femora often dusky near base; male genitalia (Fig. 21) with penis longer than ventral alae or parameres; ventral surface of penis with two raised lines separated at the base, joined just past the middle of the length to form a ridge reaching almost to the apex where the lines separate; parameres much shorter than penis, compressed dorso-ventrally, tufted with hairs at apex. Length, 2.1–2.3 mm.; width, 1.7–2.0 mm.

The color pattern of the head and pronotum are not reliable characters for separating *puncticollis* Lec. from allied species. Generally speaking, specimens from Missouri, Illinois, and Indiana have the black area of the pronotum reduced to a basal black spot which may reach half the length or the full length of the pronotum. On the other hand, Minnesota specimens usually have the black area of the pronotum so developed as to leave only the narrow lateral margins or anterior angles pale. Melsheimer's *collaris* represents the first type of pronotal pattern and the type of *puncticollis* Lec. the second type of pattern. The Melsheimer and Leconte specimens are both females. Horn (1895) first described the characters of the first ventral segment of the male.

The name *collaris* Melsh., was preoccupied by *collaris* Herbst (1797). Weise (1929) proposed *melsheimeri* as a new name, but *puncticollis* Lec. was the first valid name given the species and must stand as such.

Distribution records:

OHIO: Athens, V-2-24, Wm. C. Stehr (O.S.U.).

INDIANA: Clarke Co., V-6-09, W. S. B., Orange Co., VI-2-08, W. S. B.; Marion Co., VI-7-03, W. S. B.; Lawrence Co., V-12-09, W. S. B.; Steuben Co., VI-25-09, W. S. B.; Posey Co., V-11-03, W. S. B.; Crawford Co., V-16-03, W. S. B.; Martin Co., VII-11-03 and VII-13-03, W. S. B.; Pulaski Co., VII-5-35, D. W. LaHue; Tippecanoe Co., VI-20-35, D. W. LaHue (Purdue): Pine, V-28-05, A. B. Wolcott (C.N.H.M.).

ILLINOIS: Oakwood, VI-6-25, T. H. Frison; Shawneetown, IV-28, 29-26, Auden and Frison; Urbana (Brownfield Woods), V-6-26, T. H. Frison; E. Cape Gir., V-7-32, H. L. Dozier (I.N.H.S.).

MICHIGAN: N. Muskegon, VII-11-06, C. A. Hill (C.N.H.M.): Kalamazoo, August, Blackwelder (U. of Mich.).

WISCONSIN: Bayfield, Co., VI-26-97; Lake Namekagon (Bayfield Co.), July, 1922, Wm. S. Marshall (U. of Wis. Zoo.): Madison, C. L. Fluke; Columbus, VI-25-24, C. L. Fluke (U. of Wis. Ent.): Rib Mountain St. Park, VIII-27-37, L. R. Penner (U. of Minn.).

MINNESOTA: Plummer, VI-19-33, D. Denning; White Bear, VII-10-21, Wm. E. Hoffman; Houston Co., V-21-38, H. E. Milliron; Olmsted Co., C. N. Ainslie; St. Anthony Park, VI-5-11, Lugger Coll.; Chicago, V-16-36; Houston Co., V-23-36, H. R. Dodge; St. Paul (Battle Creek Park), VI-17-29, Wm. C. Stehr; Itasca Park, VI-17-38, H. E. Milliron; Whitefish Lake, VII-1-21, H. B. Hungerford; Laporte, VIII-1-30, D. Denning; Washington Co., VII-21-11; Cass Co., O. W. Oestlund; Pine Co., V-28-38, H. E. Milliron; Niswa, VII-4-29, O. E. Storm (U. of Minn.).

IOWA: Ames, VII-19-95, V-10-94 (I.S.C.): Bremer Co., VII-11-33: Iowa Co., VII-30-34, H. Jaques; Appanoose Co., VI-19-33, H. E. J.: Hancock Co., VII-8-33, H. E. J.; Emmet Co., VII-8-33, H. E. J.; Mt. Pleasant, VII-27-30, McGavic (I.I.S.).

MISSOURI: Atchison (WSC), Boone (WSC), Callaway (GWT), Cape Gir. (CWW), Harrison (EHF), Monroe (CWW), Perry (CWW), St. Louis (RCF), and Stone (RCF) counties; Columbia, VII-10-04, R. W. Wolcott (U. of Neb.).

KANSAS: Miami Co., 1915, R. H. Beamer (U. of Kan.).

NEBRASKA: Sioux Co. (Monroe Canyon), VI-24-11, R. W. Dawson; Lincoln, VI-30-10, F. A. Burnham (U. of Neb.).

52. *Scymnus* (*Pullus*) *uncus* new species (Pl. I, Fig. 23)

Oval, a little elongate, convex; head reddish yellow; pronotum reddish yellow with an ill-defined black spot at the base before the scutellum, not reaching the middle of the length; punctulation of pronotum shallow, not closely set; elytra black, reddish yellow at apex for 0.13 mm.; punctulation of elytra coarser and more closely set than pronotum; abdomen with first and second segments black, third, fourth, and fifth reddish yellow; first ventral abdominal segment of male with an unimpressed glabrous area at middle; margins of glabrous area of male first ventral defined by fine punctulation and pubescence; fifth ventral abdominal segment of male deeply foveate; legs reddish yellow; mesosternum and metasternum black; prosternum reddish yellow, black between the coxae; prosternal carinae convergent, complete to the anterior margin; male genitalia (Fig. 23) with penis longer than parameres, equal in length to the ventral alae; penis ending in a broad, ventrally-curved, hook-shaped process; ventral alae large, connected to the penis at three-fourths the length; parameres widest at about middle of length, apex rounded, ornamented with a tuft of hairs reaching to the ends of the ventral alae. Length, 2.2 mm.; width, 1.6 mm.

Holotype (male). County #43 (Monona Co.), Iowa, VI-30-32, Russell. Known only from the single male in the collection of the Iowa Insect Survey, Iowa Wesleyan College, Mt. Pleasant, Iowa.

This species is allied by the shape of the penis to *brullei* Muls., but is easily separated from *brullei* by the shape of the ventral alae as well as the penis. The type locality of *uncus*, Monona Co., Iowa, is situated at about the center of the western border of Iowa.

53. *Scymnus* (*Pullus*) *cultratus* new species (Fig. 24)

Oval, convex; head black, finely red before the clypeus or black at the base and reddish brown from the middle to the clypeus; pronotum black, sometimes brownish at the anterior angles; punctulation of pronotum a little more closely set at sides than on disc; elytra black with the bead finely reddish at the apex; abdomen black or dark brown; first ventral abdominal segment of males with an ill-defined, almost impunctate, glabrous area at the middle defined by long pubescence and closely set punctures; fifth ventral abdominal segment of males broadly impressed with the sixth to form a wide and rounded fovea; legs brown, femora black at base; sternum black; prosternal carinae convergent, complete to the anterior margin; male genitalia (Fig. 24) with penis longer than ventral alae; membrane joining alae and penis broadly curved toward the base; base of the penis short; penis curved along internal margins; parameres short and stubby, about one-half as long as penis, terminal hair tufts not reaching the tips of the ventral alae; ventral face of

penis with a long, median, blade-like ridge or process from near the base to the apex. Length, 2.2–2.4 mm.; width, 1.6–1.7 mm.

Holotype (male). Cook Co., Minn., Rosebush Township, Lake Superior shore, August 9, 1929, Wm. C. Stehr.

Allotype (female). Same data as holotype.

Paratypes. (1 male and 4 females). Same data as holotype and allotype: (1 male, 1 female) 6 mi. east of Holt, Minn., July 23, 1935, D. G. Denning.

This species is allied by the characters of the male genitalia to *puncticollis* Lec. The central blade-like ridge of the penis is distinctive and serves to separate *cultratus* from the latter species.

54. *Scymnus* (*Pullus*) *natchezianus* Casey (Fig. 34)

1899 *Scymnus* (*Pullus*) *natchezianus* Casey, Jour. N. Y. Ent. Soc. 7:143.
1920 *Scymnus natchezianus* Leng, Cat. Col. N. Am., p. 213.
1931 *Scymnus* (*Pullus*) *natchezianus* Korschefsky, Col. Cat. pars 118, p. 163.

Oval, rounded, convex; head yellow or yellowish red; pronotum yellowish red with a parabolic black spot extending from the base almost to the front margin; elytra black, narrowly tipped with red at apex; abdomen black, last two segments pale; first ventral abdominal segment of males with a median glabrous area at apex of segment, defined by fine and densely set pubescence; fifth ventral abdominal segment of males with a deep, rounded bevel at posterior margin; prosternal carinae convergent, complete to the anterior margin; legs brown or yellowish red; male genitalia (Fig. 34) with penis and ventral alae equal in length; penis broadly bullet-shaped dorsally; ventral surface of penis with a sharply-pointed process running along the center and projecting well beyond the broad dorsal part; ventral alae sinuate along inner margins; parameres shorter than penis and ventral alae, tufted with long hairs at apex. Length, 1.8–2.2 mm.; width, 1.5–1.7 mm.

Distribution records:

MISSOURI: Cardwell, V-12-40 (1 male and 1 female), R. C. Froeschner: Columbia, X-15-34 (U. of Mo.).

KANSAS: State record, T. B. A. collector, Blatchley Coll. (Purdue).

Casey (1899) described the species from a single male collected at Natchez, Miss. At present there are five females, two from Minnesota and three from Wisconsin labeled *natchezianus* Csy., in the Casey collection at the National Museum at Washington, D. C. Presumably, these females were labeled by Casey himself at some later date. It is questionable, in the author's mind, whether these females are *natchezianus* Casey as no males of the species have been seen from Minnesota. The Missouri and Kansas specimens recorded above fit Casey's description well, but until the genitalia of the type are dissected and examined, the true status of these specimens is doubtful.

55. *Scymnus* (*Pullus*) *lacustris* Leconte (Fig. 31)

1850 *Scymnus lacustris* Leconte in Agassiz, Lake Superior 4:239.
1852 *Scymnus lacustris* Leconte, Proc. Acad. Nat. Sci. Phila. 6:140.
1874 *Scymnus lacustris* Crotch, Rev. Cocc., p. 140.
1895 *Scymnus lacustris* Horn, Trans. Amer. Ent. Soc. 22:105.
1899 *Scymnus* (*Pullus*) *lacustris* Casey, Jour. N. Y. Ent. Soc. 7:149.
1920 *Scymnus lacustris* Leng, Cat. Col. N. Am., p. 213.
1931 *Scymnus* (*Pullus*) *lacustris* Korschefsky, Col. Cat. pars 118, p. 161.

Oval, rounded, convex; head reddish brown or yellow; pronotum entirely black, black with anterior angles pale, or reddish brown with a large parabolic black spot from base to front margin; pronotal punctures deeper and more closely set at margins than on disc; elytra black, frequently narrowly bordered with reddish brown at apex; abdomen entirely black or black basally with fourth and fifth segments reddish brown; first ventral abdominal segment of male with a glabrous area at middle surrounded by dense punctures and pubescence; fifth ventral segment of male with a deep rounded impression at posterior margin extending into the sixth segment; legs reddish brown, femora often black for two-thirds the length; prosternal carinae slightly convergent, complete to the anterior margin; male genitalia (Fig. 31) with penis as long as ventral alae; penis sharply angulate at apex; dorsal plate of penis forming an angle with the ventral alae at point of junction; ventral alae lightly sclerotized at apex; parameres shorter than penis and alae,

tipped at the apex with long hairs which reach further caudad than apex of penis. Length, 2.1-2.3 mm.; width, 1.7-1.9 mm.

Distribution records:

INDIANA: Mineral Springs, VIII-20-11 and IX-16-11 (Tamarack Swamp), A. B. Wolcott; Beverly Shores, VI-39, H. Dybas (C.N.H.M.): Steuben Co., VI-16-08, W. S. Blatchley (Purdue).

MICHIGAN: Marquette, VIII-7 (M.C.Z.): Charlevoix Co. (Beaver Island), VIII-29--22, M. H. Hatch (U. of Mich.): State record, Chope Coll. (C.N.H.M.).

MINNESOTA: Itasca Park, VI-17-38, H. E. Milliron; Houston Co., V-27-39, H. T. Peters (U. of Minn.).

As the records above indicate, *lacustris* Lec. is a northern species, and though closely related to *natchezianus* Csy., it has not been collected south of northern Indiana. Since the pronotal, pattern, leg color, and color patterns in general vary considerably within the species, positive identification of specimens is thought to be impossible without examination of the male genital characters.

Leconte (1852) described *abbreviatus* from females which can be separated from *lacustris* Lec. only by the length of the metacoxal arcs of the first ventral segment. No specimens of *lacustris* have been seen in which the metacoxal arcs are as short as those described by Leconte in the case of *abbreviatus*.

56. *Scymnus* (*Pullus*) *majusculus* new species (Fig. 30)

Oval, widely rounded, convex; head reddish yellow; pronotum black, lateral margins widely pale, punctulation sparse and inconspicuous; elytra black, narrowly tipped at apex with reddish brown, punctures not closely set, deeper than those of pronotum; pubescence of elytra coarse, erect, more sparsely set than in related species; abdomen black or dark brown basally, fifth segment reddish brown; first ventral abdominal segment of male with a small, triangular, unimpressed, glabrous area at the middle of posterior margin; glabrous area of first ventral defined by closely set punctures and long, sparse pubescence; fifth ventral abdominal segment of male with a deeply sinuate posterior margin; sternum black; prosternal carinae complete to the front margin, convergent for two-thirds the length, divergent through anterior one-third; legs reddish yellow, femora dusky at base; male genitalia (Fig. 30) with penis shorter than ventral alae; penis very broad at point of attachment with ventral alae; ventral internal margins of penis forming a trough-shaped apical process notched at distal end; ventral alae heavily sclerotized and pigmented as far as the apex of penis, extending beyond the penis as membranous lobes; parameres rising well dorsad of the base of the alae, much shorter than penis and alae; apical hairs of parameres reaching caudad to the distal ends of the alae. Length, 2.7 mm.; width, 2.0 mm.

Holotype (male). Hessville, Ind., VII-4-06, W. J. Gerhard in the collection of the Chicago Natural History Museum.

Considering the form of the penis and parameres of the male genitalia, *majusculus* seems only distantly related to other species of the subgenus. The large size of the single specimen seen is distinctive, and may well prove to be a valid character to distinguish the species if additional specimens show little variation in respect to size. Of the 117 forms (species and subspecies) of *Scymnus* known to Casey (1899), only two, *Scymnus* (*Pullus*) *haemorrhous* var. *laurenticus* Casey and *Scymnus* (*Scymnus*) *americanus* Muls. were listed as being 2.7 mm. in length.

57. *Scymnus* (*Pullus*) *tenebrosus* Mulsant (Fig. 27)

1850 *Scymnus* (*Pullus*) *tenebrosus* Mulsant, Spec. Trim. Sécuripalp., p. 989.
1852 *Scymnus tenebrosus* Leconte, Proc. Acad. Nat. Sci. Phila. 6:140.
1874 *Scymnus tenebrosus* Crotch, Rev. Cocc., p. 268.
1895 *Scymnus tenebrosus* Horn, Trans. Amer. Ent. Soc. 22:106.
1899 *Scymnus* (*Pullus*) *tenebrosus* Casey, Jour. N. Y. Ent. Soc. 7:148.
1899 *Scymnus* (*Pullus*) *compar* Casey, Jour. N. Y. Ent. Soc. 7:148.
1910 *Scymnus tenebrosus* Blatchley, Indiana Dept. Geol. Bull. 1, p. 531.
1920 *Scymnus tenebrosus* Leng, Cat. Col. N. Am., p. 213.
1920 *Scymnus compar* Leng, Cat. Col. N. Am., p. 213.
1930 *Scymnus tenebrosus* Stehr, Minn. Agr. Exp. Sta. Tech. Bull. 75, p. 50.
1931 *Scymnus tenebrosus* Korschefsky, Col. Cat. pars 118, p. 156.
1931 *Scymnus* (*Pullus*) *compar* Korschefsky, Col. Cat. pars 118, p. 156.

Oval, convex; head yellow, pale brown, or black in males; head of females black with clypeus pale brown or red, rarely entirely pale; pronotum entirely black or

reddish brown with a basal black spot variable in size; elytra entirely black or black with a very narrow apical pale border; abdomen black, the last three segments sometimes pale; first ventral abdominal segment of males with a median glabrous area at posterior margin; glabrous area of male first ventral neither impressed nor distinctly outlined by heavier pubescence or closely set punctures; fifth ventral abdominal segment of males broadly, not deeply sinuate at posterior margin; sternum black; prosternal carinae slightly convergent, complete to the anterior margin; legs yellowish red to brown; male genitalia (Fig. 27) with penis shorter than ventral alae; penis elongate-oval dorsally, ventrally with elevated ridges joining to form a slender pointed process projecting beyond the dorsal part; ventral alae heavily sclerotized, black in the wholly black specimens, broadly expanded at apex; parameres shorter than penis, ornamented apically with tufts of long hairs rising from the ventral margin. Length, 1.9–2.3 mm.; width, 1.4–1.7 mm.

Distribution records:

OHIO: Hocking Co., IV-17-38, V-1-38, D. J. and J. N. Knull (O.S.U.).

INDIANA: Knox Co., VII-11-29, Montgomery (U. of Mich.): Lake Co., VII-14-09, W. S. Blatchley; Hessville, VII-4-08, A. B. Wolcott; Harrison Co., VI-27-34, D. W. LaHue (Purdue): Dune Park, VII-29-40, John Retondo (C.N.H.M.).

ILLINOIS: Park Ridge, VI-5-42, J. A. Slater (I.S.C.): Dubois, VIII-21-09: Anna, V-25-07; Putnam Co., V-28-33, M. O. Glenn (I.N.H.S.).

MICHIGAN: Escanaba and Marquette (M.S.C.): Charlevoix Co. (Beaver Island), VIII-22-23, IV-9-22, M. H. Hatch; Cheboygan Co. (Douglas Lake), V-27-39, I. J. Cantrall (U. of Mich.); Cheyboygan Co., 1935, H. B. Hungerford; Emmet Co., VII-23-35, Milton Sanderson (U. of Kan.).

MINNESOTA: Itasca Park, VI-16-38, H. E. Milliron; Ramsey Co. (Lake Vadnais), VI-20-29, Wm. C. Stehr; Grand Marais (Devil's Track River), VIII-7-29, Wm. C. Stehr; Olmsted Co., C. N. Ainslie; Two Harbors, VI-28-27, M. H. Hatch; Viking, VII-23-35, D. Denning; Winton (Basswood Lake), VIII-22-26, A. McIntosh; Rosseau, VI-27-20, J. P. Jensen; Walker, VI-2-35, D. G. Denning; St. Anthony Park, Lugger Coll.; Whitefish Lake, VII-1-21, H. B. Hungerford (U. of Minn.).

MISSOURI: Butler (CWW), Cape Gir. (CWW), Carter (RCF), Franklin (RCF), Iron (EHF), Shannon (RCF), and St. Louis (RCF) counties.

NEBRASKA: Louisville, V-20-11, R. H. Wolcott (U. of Neb.).

This species is closely allied to *iowensis* Csy. and *consobrinus* Lec. by the characters of the male genitalia. Mulsant (1850) described *tenebrosus* from wholly black specimens. Subsequent collections of the species have yielded many specimens with pale areas on the pronotum and elytra. The male genitalia of these forms are identical with the genitalia of black specimens. In many cases the females have the head black and clypeus pale, but as Horn (1895) has indicated, the color of the head is not always an indication of sex. Casey (1899) described *compar* from a single female collected in Indiana. The differences between *tenebrosus* Muls. and *compar* Csy. noted by Casey are not clearly seen in Casey's type of *compar*. Without associated males with distinctive genital characters differing from those of *tenebrosus* Muls., it seems best to consider *compar* Csy. a synonym of *tenebrosus* Muls. Wilson (1927) figured the male genitalia of a specimen identified by him as *tenebrosus* Muls. The illustration fails to show the distinctive long and slender ventral process of the penis found in *tenebrosus* Muls.

58. *Scymnus* (*Pullus*) *iowensis* Casey (Fig. 28)

1899 *Scymnus* (*Pullus*) *iowensis* Casey, Jour. N. Y. Ent. Soc. 7:143.

Oval, rounded, convex; head yellow to reddish brown; pronotum yellow to reddish brown with a central black area variable in size and form; central black area of pronotum usually a parabolic black spot or rectangular black area reaching from the base almost to the front margin of the pronotum; elytra entirely black or narrowly margined with reddish brown; abdomen black, frequently with one, two, or three terminal segments pale; first ventral abdominal segment of males with a central, oval or triangular glabrous area, glabrous area of male first ventral depressed so as to appear slightly concave, defined by dense pubescence and closely set punctures; fifth ventral abdominal segment of male with a broadly rounded sinus formed by the sinuate margin of the fifth segment and an impressed

area of the sixth segment; prosternal carinae convergent, complete to the anterior margin; male genitalia (Fig. 28) with penis shorter than ventral alae; penis widely rounded dorsally, ventrally with raised convergent ridges uniting to form a projecting process as in *tenebrosus* Muls.; apical process of penis shorter, broader, and more rounded than in *tenebrosus* Muls., slightly curved dorsad; ventral alae heavily sclerotized and pigmented except at the ends which are broadly expanded into thin, rounded, plate-like lobes projecting toward the penis, length, 2.0–2.3 mm.; width, 1.5–1.7 mm.

Distribution records:

INDIANA: Knox Co., VII-11-29, Montgomery (U. of Mich.); Lafayette, VII-5-05, A. B. Wolcott (C.N.H.M.); Brown Co., VI-15-27, W. S. Blatchley; Jasper Co., VII-4-32, D. W. LaHue; Tippecanoe Co., VI-20-35, D. W. LaHue (Purdue).

ILLINOIS: Algonquin, VI-1, 11-07, Nason in Fall Coll. (M.C.Z.); N. Ill., June, Peabody Coll.; Ill. Bolter Coll. (I.N.H.S.); Chicago, VI-20-11, Wolcott; W. Pullman, V-10-03, W. J. Gerhard (C.N.H.M.).

MICHIGAN: Oakland Co., VII-4-24, W. Andrews, Fall Coll. (M.C.Z.); Pentwater, VII-13-20, E. Liljeblad; Oakland Co., VI-22-21, T. H. Hubbell (U. of Mich.); State record, Chope Coll. (C.N.H.M.); Otsego Co., IV-8-35, (U. of Kan.).

WISCONSIN: State record, Bolter Coll. (I.N.H.S.): Lakewood, IX-16-46, H. E. Milliron (U. of Minn.).

MINNESOTA: State record (C.N.H.M.); Houston Co., V-21-38, P. Nicholson; Douglas Co., VI-14-37, D. G. Denning; St. Anthony Park (Breeding Cage #1186) VII-28-13; Owatoma, VI-23-22, Wm. E. Hoffman; Hennepin Co., C. W. Oestlund; Minneapolis, V-24-29, Wm. C. Stehr; Laporte, VII-4-35, D. G. Denning; Olsteed Co., C. N. Ainslie; Climax, VII-8-35, D. G. Denning; Two Harbors, VI-28-27, M. H. Hatch; Tamarack, VI-10-34, D. G. Denning (U. of Minn.).

IOWA: Shenandoah, V-3-46, H. B. Green; Ames, VIII-11-12, D. Stone; Boone, VII-7-33, F. Andre (I.S.C.); Mt. Pleasant, V-22-28, Berry (C.N.H.M.); Jefferson Co., V–24–33, Edwards; Cass Co., VII–24–33, H. E. Jaques, 35, L. C. W.; Blackhawk Co., VIII–8–33; Wapello Co., VI–14–35, L. Cutkamp (I.I.S.).

KANSAS: Miami Co., 1925, R. H. Beamer (U. of Kan.).

NEBRASKA: Lincoln, VII-18-08, C. H. Gable (U. of Neb.); Valentine, VI-11050, Hicks, Slater, Laffoon (I.S.C.).

59. *Scymnus* (*Pullus*) *consobrinus* Leconte (Fig. 29)

1852 *Scymnus consobrinus* Leconte, Proc. Acad. Nat. Sci. Phila. 6:139.
1852 *Scymnus consobrinus* Mulsant, Ann. Soc. Agr. Lyon 1:153.
1874 *Scymnus consobrinus* Crotch, Rev. Cocc., p. 266.
1895 *Scymnus consobrinus* Horn, Trans. Amer. Ent. Soc. 22:103.
1899 *Scymnus* (*Pullus*) *consobrinus* Casey, Jour. N. Y. Ent. Soc. 7:142.
1920 *Scymnus consobrinus* Leng, Cat. Col. N. Am., p. 213.
1930 *Scymnus consobrinus* Stehr, Minn. Agr. Exp. Sta. Tech. Bull. 75, p. 50.
1931 *Scymnus consobrinus* Korschefsky, Col. Cat. pars 118, p. 157.

Oval, rounded, convex; head yellow or yellowish red; pronotum yellow or yellowish red with a median black spot broad at base, reaching past the middle of the length; basal black spot of pronotum of females often expanded leaving only the lateral margins pale; elytra black, finely tipped with the pale color at the apex; abdomen black, fourth and fifth segments yellowish red; first ventral abdominal segment of males with a small deeply-impressed glabrous area at posterior margin; glabrous area of male first ventral surrounded by pubescence and closely-set punctures placed on the raised edge of the area and accentuating the outline; fifth ventral abdominal segment of males with a deep, rounded fovea at posterior margin extending into the sixth segment; prosternal carinae convergent, complete to the front margin; male genitalia (Fig. 29) with penis a little shorter than ventral alae; penis spade-shaped dorsally, ventral surface with a raised process formed by two convergent ridges ending in a blunt point opposite the tip of the dorsal part of the penis; ventral alae broadly expanded, thin and leaf-like at the ends; parameres almost as long as penis, ornamented with a row of hairs along the distal one-sixth of the length. Length, 1.9–2.3 mm.; width, 1.4–1.7 mm.

Distribution records:

INDIANA: Miller, VI-4-14, A. B. Wolcott (C.N.H.M.).

ILLINOIS: Willow Springs, VI-18-16, VI-17-15 (U. of Mich.): State records, Bolter Coll.; Normal, V-5-82; Pulaski, VIII-10-09; Algonquin, VIII-8-96 (I.N. H. S.): Willow Springs, VI-26-07, E. B. Chope; Palos Park, VII-19-12, Wolcott; Beverly Hills, V-22-04, W. J. Gerhard (C.M.H.N.).

WISCONSIN: Elkhorn, VII-18-89 (I.N.H.S.).

IOWA: McGregor, VII-15-04, E. Liljeblad (U. of Mich.): Sioux City, October and November, 1935, C. N. Ainslie (U. of Minn.).

The present position of *consobrinus* Lec. is not clear. There is no type in the Leconte collection. An empty pin with the label, "*S. consobrinus* Lec. – *caudalis* Lec. – *fastigiatus* Muls." remains. Wilson (1927) figured the male genitalia of a specimen which was said to have been compared with the type. However, it is the author's opinion that the genitalia figured by Wilson are those of a specimen of *puncticollis* Lec. and not *consobrinus* Lec. The specimens recorded above fit the original description of Leconte (1852) and the description of Casey (1899). In the latter, Casey notes particularly the deeply impressed glabrous area of the male first ventral, and refers to it as a pit at the apical margin.

60. *Scymnus* (*Nephus*) *intrusus* Horn. — A small, pale brown species seen from Indiana, Illinois, Iowa, Missouri, and Kansas.

61. *Scymnus* (*Nephus*) *flavifrons* Melsh. — A not uncommon species but never abundant in the region. Records from: Ohio, Indiana, Illinois, Minnesota, Iowa, Missouri, Kansas, and Nebraska.

62. *Scymnus* (*Nephus*) *ornatus* Leconte. — Described from the north shore of Lake Superior by Leconte (1850), *ornatus* seems to be a northern species. Records at hand are from Wisconsin and Minnesota.

63. *Scymnus* (*Diomus*) *ohioensis* Stehr. — Known only from the single female collected in Gallia Co., Ohio.

64. *Scymnus* (*Diomus*) *amabilis* Leconte. — A single specimen of this rare species has been seen: West Okoboji Lake, Iowa, IX-3-49, Jean Laffoon. This is not the *amabilis* described and figured by Horn (1895). Horn's figure agrees neither with Leconte's description nor with the type. Leconte (1852) did not indicate the extent of the metacoxal plates other than that they were incomplete externally. The specimen figured by Horn as *amabilis* has the metacoxal plates peculiar to the subgenus *Nephus* Muls., while the type of *amabilis* Lec. has metacoxal plates typical of the subgenus *Diomus* Muls.

65. *Scymnus* (*Diomus*) *quadritaeniatus* Leconte. — Specimens from Illinois, Iowa, and Missouri have been seen. The species evidently hibernates in small colonies as Mr. W. S. Craig reports having taken it in numbers under bark during late winter and early spring in Missouri.

66. *Scymnus* (*Diomus*) *liebecki* Horn. — A very rare species described by Horn (1895) from Elkhart, Indiana. A single specimen from St. Clair Co., Ill., VI-20-03, G. W. Bock, is in the Bock collection at the University of Missouri.

67. *Scymnus* (*Diomus*) *xanthaspis* Mulsant. — Although this has been considered a southern species, records are available from Indiana, Illinois, and Missouri. The Illinois records are from the northern section of the state.

68. *Scymnus* (*Diomus*) *terminatus* Say. — Common and frequently abundant in Ohio, Indiana, Illinois, Iowa, Missouri, and Kansas. It is frequently associated with *Sorghum halepense* (L.) in Missouri.

69. *Scymnus* (*Diomus*) *dulcis* Casey. — Casey described this species from Kansas in 1899. I have seen no example of it except the type. The type is labeled simply 'Ks' and it is possible that *dulcis* is a western species ranging westward from the 100th meridian.

70. *Scymnus* (*Diomus*) *aeger* Casey. — No specimens of this species have been seen except the types in the Casey collection from Marquette, Michigan, and a paratype from Illinois. An examination of the type of *molliculus* Csy. (1924) revealed no distinctive differences with *aeger*. I therefore consider *molliculus* a synonym of *aeger*.

In the following, the genus *Nephaspis* Casey is recorded for North America for the first time. It seems advisable to insert the following notes concerning the genus.

Genus *Nephaspis* Casey

1899 *Nephaspis* Casey, Jour. N. Y. Ent. Soc. 7:168.
1905 *Nephaspis* Casey, Can. Ent. 37:161.
1931 *Nephasis* Korschefsky, Col. Cat. pars 118, p. 168.
1945 *Nephasis* Blackwelder, Smithson, Inst. Bull. 185, p. 445.

Head large, deeply inserted into thorax; front well developed in front of eyes; clypeus large, deeply emarginate; antennae with first two segments large and flattened, remaining segments, except club, small and slender; pubescence of elytra fine; epipleurae narrow, obsolete just behind base of abdomen; sternum convex; posterior margin of metasternum excavate for reception of hind legs; anterior margin of mesosternum abruptly truncate; middle of prosternum and front coxae hidden by head in repose; front coxae slender, conical, widely separated at base, contiguous at apex; tarsal claws slender, simple.

The genus *Nephaspis* was erected by Casey (1899) when *Nephaspis gorhami* was described. In his remarks upon the salient characters of the genus, Casey noted that the metacoxal arcs were nearly as in the subgenus *Nephus* Muls. An examination of the types of *gorhami* Csy. has revealed that the metacoxal arcs more nearly resemble those of the subgenus *Scymnus* Kug. This was also noted in the case of the new species of *Nephaspis* described from Iowa in the present work. As a matter of fact, there is considerable variation in the extent of recurvature of the metacoxal lines in the various specimens. In some cases the line is only slightly recurved, as in the subgenus *Scymnus*, while in others the line is recurved and extended almost to the base of the abdomen. Without close inspection, in some cases involving removal of the abdomen in extracting genitalia, the metacoxal arcs in the latter may seem complete as in the subgenus *Pullus* Muls.

Up to the present time the genus has been represented in the world fauna by a single species, *Nephaspis gorhami* Csy., described from Colombia in 1899. In the same paper Casey described *brunnea* which he later (1905) designated as the female of *gorhami* Csy. A new species collected in central Iowa is added to the genus by the following description.

71. *Nephaspis amnicola* new species (Figs. 32 and 33).

Oval, convex; head yellow; pronotum entirely yellow or yellow with a median hour-glass-shaped dark brown area from the base to the anterior margin; punctulation of pronotum faint, not closely set; elytra black at base and along lateral margins; central area of elytra testaceous, apex narrowly yellow; punctulation of elytra coarse, much more distinct and closely placed than that of pronotum; pubescence of elytra fine, suberect, directed longitudinally, not directed in the whorled pattern usual in the genus *Scymnus* Kug.; abdomen black or dark brown except segments five and six and narrow lateral margins of three and four pale; metacoxal arcs of first ventral abdominal segment incomplete, recurved toward base of abdomen, variable in extent; area enclosed by metacoxal lines pubescent in a straight line along basal margin, widely glabrous along curved posterior margin; meso- and metasternum very convex, coarsely punctulate; prosternum yellow; legs yellow; male genitalia (Figs. 32 and 33) with basal plates long; penis sharply pointed at apex; parameres rising from behind penis when seen from ventral aspect, broadly curved from base of penis when viewed from lateral aspect. Length, 1.3–1.4 mm.; width, 0.9–1.0 mm.

Holotype (male). Ledges State Park (Boone, Iowa), IX-16-49, C. W. Wingo.

Paratypes (2 males). Ledges State Park (Boone, Iowa), VII-4-50, C. W. Wingo; IX-29-50, Jean Laffoon. All types in the collection of the author.

The holotype was taken while sweeping herbage and shrubs in a valley near a small stream. The first paratype listed above was taken in the identical spot somewhat less than a year later. An unsuccessful attempt was made at that time to determine the food and associated plants of the species. Mr. Laffoon took the second paratype in a neighboring valley later in the year while doing general sweeping. The vicinity of the type locality is an unusual situation not usually found in central Iowa. Large limestone bluffs tower along the valleys through which shallow, clear streams flow. In general, the area resembles the valleys found in the Missouri Ozarks with the plant life much the same.

Nephaspis amnicola nob. resembles *gorhami* Csy. closely, but is slightly larger in size and differs somewhat in the color of the upper surface, *gorhami* Csy. having the pronotum entirely yellow and the elytra black. However, as color patterns are notoriously poor characters in the tribe *Scymnini* there is a possibility that the

two species may actually be the same. The widely separated points of collection of the two forms make this possibility most unlikely as few of the North American species of the family occur in South America.

72. *Cephaloscymnus zimmermanni* Crotch. — There is a single record for this southern species in the Upper Mississippi Basin: Knox Co., Ind., VII-2-03, W. S. Blatchley.

73. *Cryptolaemus montrouzieri* Mulsant. — It is likely that this Australian species has spread from the original introduction point in California to the midwestern states in shipments of citrus fruits. The following records are available: Lafayette, Ind., V-21-35 (in bush), H. E. Brown; Washington, Mo., VII-13-38, W. R. Enns; state records for Missouri in student collections, U. of Mo.

74. *Delphastus pusillus* (Leconte). — This very small and compact beetle seems to be common in Missouri, Illinois, and the states to the south. Blatchley (1910) listed *pusillus* as common in Indiana, but there are no specimens from Indiana in the Blatchley collection. Records are available from the following states; Ohio, Indiana, Illinois, Iowa, and Missouri.

75. *Coccidula lepida* Leconte. — Generally restricted to the northern states recorded below. Records from Ohio, Indiana, Illinois, Michigan, Wisconsin, Minnesota, and Iowa.

76. *Coccidula suturalis* Weise. — Weise originally described *suturalis* as a variety of *lepida* (Lec.). I follow Dodge (1938) in giving *suturalis* specific rank. It is likely that a study of adequate material will place *suturalis* as a well-defined subspecies of *lepida*. Records are available for Illinois, Wisconsin, and Minnesota.

77. *Psyllobora vigintimaculata* (Say). — A very common species in the Upper Mississippi Basin and recorded from all states.

78. *Anisosticta bitriangularis* (Say). — A northern species which is very occasionally collected as far south as central Iowa and Illinois. Recorded from Indiana, Illinois, Michigan, Wisconsin, Minnesota, Iowa, and Nebraska.

79. *Macronaemia episcopalis* (Kirby). — Essentially boreal in distribution, this species is seldom found even in the northern states of the Upper Mississippi Basin. I have seen specimens from Minnesota, Iowa, and Kansas.

80. *Coleomegilla maculata lengi* Timberlake. — This is the familiar *Ceratomegilla* or *Megilla fuscilabris* of authors. Common and abundant throughout the area.

81. *Hippodamia tredecimpunctata tibialis* (Say). — Common throughout the upper two-thirds of the region studied. Very scarce below the latitude of St. Louis, Mo.

82. *Hippodamia americana* Crotch. — Not seen by the author. Chapin (1946) reported a single male in the National Museum taken by Hubbard and Schwarz on Whitefish point, Lake Superior, Mich.

83. *Hippodamia parenthesis* (Say). — Frequently collected throughout the area studied except in the southern half of Missouri and the southern tips of Illinois and Indiana, where it seldom occurs.

84. *Hippodamia quinquesignata* (Kirby). — Rare in the Upper Mississippi Basin. The following records extend the southern limits of the range of the species as given by Chapin (1946): Indiana, Michigan, Minnesota, Iowa, and Nebraska.

85. *Hippodamia glacialis* (Fab.). — This species tends to be slightly more plentiful in the northern part of the Basin. For instance, it is not uncommon in central Iowa, but is scarce in Missouri. Recorded from Ohio, Indiana, Illinois, Michigan, Minnesota, Iowa, Missouri, Kansas, and Nebraska.

86. *Hippodamia convergens* Guérn. — The most common species of the family found in the Upper Mississippi Basin. During the winter months *convergens* congregates in large masses in sheltered grass clumps. The largest mass seen by the author was collected in Missouri in a clump of Little Bluestem grass five inches in diameter. This grass clump held a total of 447 beetles, 445 of which were *convergens*. The other two specimens were *Hippodamia parenthesis* (Say).

87. *Hippodamia quindecim-maculata* Mulsant. — Recorded during the present study from Ohio, Indiana, Illinois, Michigan, Wisconsin, Minnesota, Iowa, Missouri, Kansas, and Nebraska. The following southern records should be noted: Tallulah, La., VII-19-30, P. A. Glick, Fall collection, Museum of Comparative Zoology; Southwest Arkansas, Chas. Palm, American Museum of Natural History. The above records extend the range of the species as given by Chapin (1946) eastward to Ohio and southward to Louisiana.

88. *Neoharmonia venusta* (Melsh.). — The melanic form (*notulata* Muls.) is included here. The male genitalia of the two forms are identical and the two are

collected in company. Recorded from Ohio, Indiana, Illinois, Michigan, Iowa, Missouri, Kansas, and Nebraska.

89. *Coccinella novemnotata* Herbst. — This is the only species of the genus *Coccinella* which occurs in abundance throughout the Upper Mississippi Basin.

90. *Coccinella californica* Mannerheim. — Specimens from Ames, Iowa, Mt. Pleasant, Iowa, Henry Co., Iowa, and Columbia, Mo. have been seen. These are the first known records of the species occurring east of Arizona. It is probable that the species has spread from the west with fruit and vegetable shipments.

91. *Coccinella transversoguttata* Fald. — The distribution is generally northern and perhaps western. Recorded from Illinois, Michigan, Iowa, Kansas, Wisconsin, and Minnesota.

92. *Coccinella nivicola monticola* Mulsant. — Recorded from Ohio, Michigan, and Minnesota. This subspecies probably occurs in Wisconsin but no specimens have been seen.

93. *Coccinella trifasciata* L. — Resembles *transversoguttata* Fald. superficially but is smaller in size and has white metepimera. Records are available for Ohio, Indiana, Illinois, Michigan, Wisconsin, Minnesota, Kansas, and Nebraska.

94. *Coccinella hieroglyphica trisuspis* Kirby. — The basal tricuspid fascia of the elytra is distinctive. This is a northern form represented in the region studied by specimens from Illinois, Michigan, Wisconsin, and Minnesota.

95. *Cycloneda munda* (Say). — This common species occurs in all states of the Upper Mississippi Basin, but seems restricted in the northern states at least as far as numbers are concerned, to the southern portions.

96. *Cycloneda sanguinea* (L.). — The pronotum of *sanguinea* usually differs from that of *munda* in having two isolated white spots on either side of the disc. Since this is not always the case, the genitalia of either sex may be used to separate the species (see figured genitalia). *C. sanguinea* is definitely a southern species. It occurs sparingly in the United States south of the state of Missouri. A single specimen was collected by the author at Advance, Mo., VIII-6-41.

97. *Olla abdominalis* (Say). — The adults and larvae of this species are often abundant on maple in September feeding on aphids which infest the maple leaves just prior to frost. Recorded from Indiana, Illinois, Wisconsin, Minnesota, Iowa, Missouri, Kansas, and Nebraska.

98. *Olla abdominalis plagiata* Casey. — Occurs in company with the typical form of *abdominalis* in Indiana, Illinois, Iowa, Missouri, Kansas, and Nebraska. This subspecies is said to make up the total population in the vicinity of Washington, D. C.

99. *Adalia bipunctata* (L.). — This familiar species occurs in all states of the of the region studied.

100. *Adalia frigida* (Schn.). — The following names are included under frigida as synonyms: *humeralis* Say, *disjuncta* Rand., *ophthalmica* Muls., *melanopleura* Lec., *annectans* Cr., *ornatella* Csy. The work of Palmer (1911) on heredity in the genus indicates that *frigida* occurs in a number of genetic forms or color phases which are not subspecies or geographic races. Records are at hand from Ohio, Illinois, Michigan, Wisconsin, Iowa, Kansas, and Nebraska. In general, *frigida* is collected frequently in the extreme northern edge of the region studied, in Canada, and in western Kansas and Nebraska.

101. *Cleis picta* (Randall). — Casey's *hudsonica* may be considered a synonym of *picta* (Rand.). The distribution of *picta* is generally northern, although occasional specimens are collected in the southern states of the region. Recorded from Ohio, Indiana, Illinois. Michigan, Wisconsin, Minnesota, Iowa, Missouri, and Kansas.

102. *Anisocalvia duodecim-maculata* (Gebler). — A boreal species, records of which are available from the states of Michigan, Wisconsin, and Minnesota.

103. *Anisocalvia quatuordecimguttata* (L.). — Another boreal species seen from Michigan and Minnesota.

104. *Anatis quindecimpunctata* (Olivier). — Found in all states of the region. It is by far the most common species of the genus in the southern half, but is replaced by *ocellata mali* (Say) in the north.

105. *Anatis ocellata mali* (Say). — This form may well prove to be a distinct species peculiar to North America instead of a subspecies of the European *ocellata*. The range is northern although occasional specimens rarely occur in Missouri and Illinois. Recorded from Indiana, Illinois, Michigan, Wisconsin, Minnesota, and Missouri.

106. *Anatis rathvoni lecontei* Casey. — Both this subspecies of *rathvoni* Lec. and

the typical form are western in distribution. A single specimen of the subspecies has been seen: Lincoln, Neb., V–2–89, T. Williams.

107. *Neomysia pullata* (Say). — Stehr (1930) reported this species confined to the northern portion of Minnesota and associated with pines infested with *Lecanium* scale and pine aphids. The species also occurs as far south as southern Missouri. Recorded from Ohio, Indiana, Illinois, Michigan, Wisconsin, Minnesota, Iowa, and Missouri.

108. *Neomysia randalli* Casey. — A rare form which may prove to be a subspecies of *pullata* (Say) when a larger material is available for study. The specimens I have seen came from Michigan, Wisconsin, and Minnesota.

109. *Neomysia horni* (Crotch). — There is a single record for this western species in the Upper Mississippi Basin: Lincoln, Neb., VIII-9-37, A. B. Shelan (U. of Neb. Coll.)

110. *Axion tripustulatum* (DeGeer). — Never common or abundant in the region studied. Records for Indiana, Wisconsin, Minnesota, Iowa, Missouri, Kansas, and Nebraska.

111. *Axion plagiatum* (Olivier). — No specimens of this species from the Upper Mississippi Basin have been seen during this study. The species is included since Blatchley (1910) listed it for Indiana, and Nunenmacher (1911) evidently had a specimen of *plagiatum* in hand when he described *incompletus*, from Illinois. Both the Indiana and Illinois specimens were collected by Wolcott within a short distance of Chicago.

112. *Chilocorus bivulnerus* Mulsant. — A common species in all of the region studied except perhaps in the northern sections of Minnesota, Wisconsin, and Michigan.

113. *Exochomus marginipennis* (Leconte). — The several named varieties seem no more than intraspecific aberrations (*childreni* Muls., *latiusculus* Csy., and *deflectens* Csy.). Recorded for Ohio, Indiana, Illinois, Missouri, and Kansas.

114. *Brumus davisi* (Leng.). — Several records are available for Wisconsin and Minnesota. The normal range of *davisi* lies westward from these states. Two closely allied species, *aethiops* (Bland.) and *septentrionis* (Wse.), may occur very rarely in Minnesota and in Nebraska east of the one hundredth meridian.

115. *Epilachna borealis* (Fab.). — The squash ladybeetle is never abundant in the Upper Mississippi Basin. Records are available for Ohio, Indiana, Illinois, Wisconsin, Minnesota, Missouri, and Kansas.

116. *Epilachna varivestis* Mulsant. — By 1947, when Professor Fluke reported the Mexican bean beetle in Wisconsin this pest had been reported in all of the Upper Mississippi Basin except eastern Kansas and Nebraska. I have seen no specimens from these states or from western Missouri or Iowa. Low relative humidity may be a limiting factor in the spread and establishment of this species.

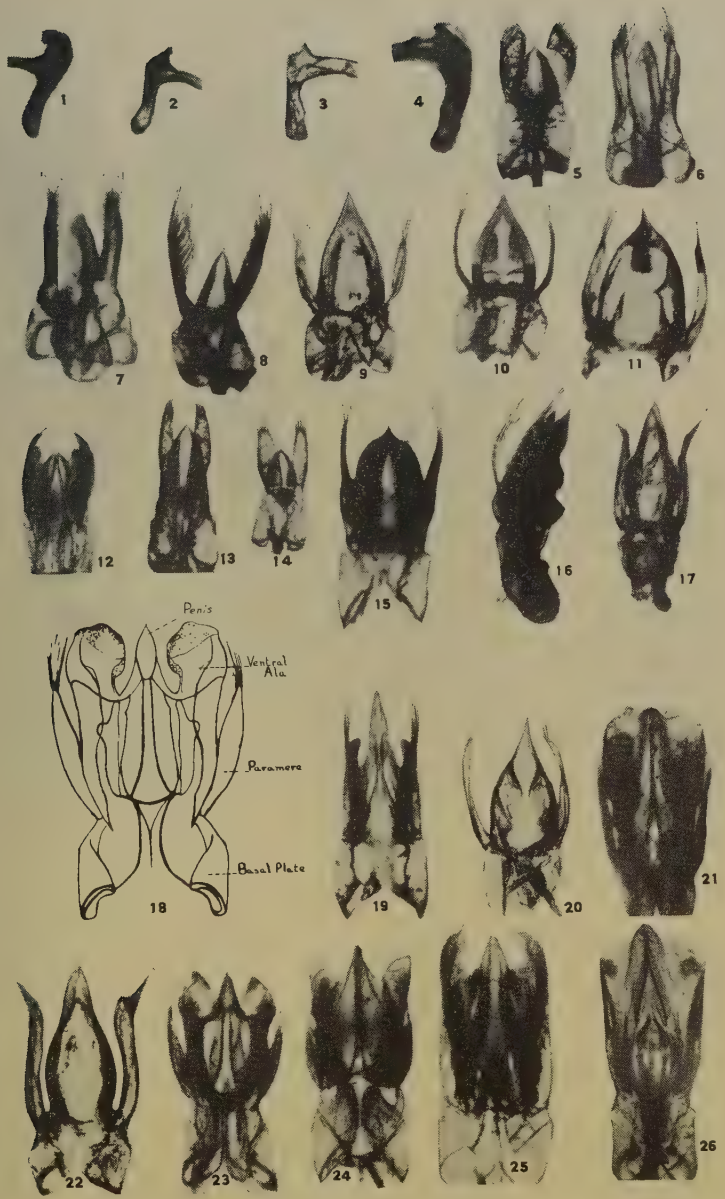
ACKNOWLEDGEMENT

The author is deeply indebted to Dr. H. H. Knight for his guidance and counsel during the course of this study and to Dr. H. M. Harris for working facilities and many other favors.

PLATE I

FIG.

1. *Scymnus* (*Pullus*) *nanus* Lec., siphonal capsule.
2. *Scymnus* (*Pullus*) *punctatus* Melsh., siphonal capsule.
3. *Scymnus* (*Pullus*) *kansanus* Csy., siphonal capsule.
4. *Scymnus* (*Scymnus*) *americanus* Muls., siphonal capsule.
5. *Scymnus* (*Pullus*) *nanus* Lec., ventral view of aedeagus.
6. *Hyperaspidius transfugatus* Csy., ventral view of aedeagus.
7. *Hyperaspidius vittigerus* (Lec.), ventral view of aedeagus.
8. *Scymnus* (*Scymnus*) *americanus* Muls., ventral view of aedeagus.
9. *Scymnus* (*Pullus*) *cinctus* Lec., ventral view of aedeagus.
10. *Scymnus* (*Pullus*) *marginicollis* Mann., ventral view of aedeagus.
11. *Scymnus* (*Pullus*) *fraternus* Lec., ventral view of aedeagus.
12. *Scymnus* (*Pullus*) *impunctus* new species, ventral view of aedeagus.
13. *Scymnus* (*Pullus*) *kansanus* Csy., ventral view of aedeagus.
14. *Scymnus* (*Pullus*) *punctatus* Melsh., ventral view of aedeagus.
15. *Scymnus* (*Pullus*) *nemorivagus* new species, ventral view of aedeagus.
16. *Scymnus* (*Pullus*) *nemorivagus* new species, lateral view of aedeagus.
17. *Scymnus* (*Pullus*) *pulvinatus* new species, ventral view of aedeagus.
18. *Scymnus* (*Pullus*) *uncus* new species, ventral view of aedeagus (trabes and sipho removed) illustrating conventional nomenclature used.
19. *Scymnus* (*Pullus*) *cervicalis* Muls., ventral view of aedeagus.
20. *Scymnus* (*Pullus*) *rubricaudus* Csy., ventral view of aedeagus.
21. *Scymnus* (*Pullus*) *puncticollis* Lec., ventral view of aedeagus.
22. *Scymnus* (*Pullus*) *festatus* new species, ventral view of aedeagus.
23. *Scymnus* (*Pullus*) *uncus* new species, ventral view of aedeagus.
24. *Scymnus* (*Pullus*) *cultratus* new species, ventral view of aedeagus.
25. *Scymnus* (*Pullus*) *brullei* Muls., ventral view of aedeagus.
26. *Scymnus* (*Pullus*) *hortensis* new species, ventral view of aedeagus.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Ventral Ala
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Basal Plate
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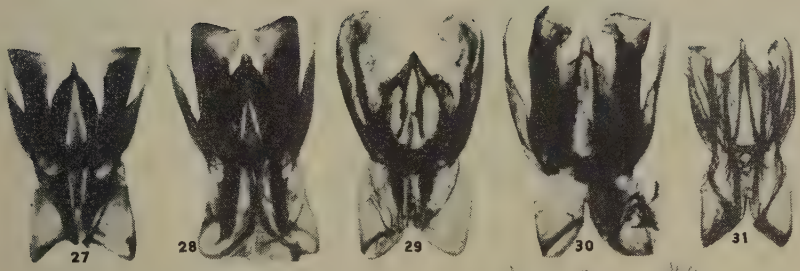
PLATE II

FIG.

27. *Scymnus* (*Pullus*) *tenebrosus* Muls., ventral view of aedeagus.
28. *Scymnus* (*Pullus*) *iowensis* Csy., ventral view of aedeagus.
29. *Scymnus* (*Pullus*) *consobrinus Lec.*, ventral view of aedeagus.
30. *Scymnus* (*Pullus*) *majusculus* new species, ventral view of aedeagus.
31. *Scymnus* (*Pullus*) *lacustris* Lec., ventral view of aedeagus.
32. *Nephaspis amnicola* new species, siphonal capsule.
33. *Nephaspis amnicola* new species, ventral view of aedeagus.
34. *Scymnus* (*Pullus*) *natchezianus* Csy., ventral view of aedeagus.
35. *Cycloneda munda* (Say), penis and parameres of male genital system.
36. *Cycloneda sanguinea* (L.), penis and parameres of male genital system.
37. *Cycloneda munda* (Say), sclerotized portion of female genital system.
38. *Cycloneda sanguinea* (L.), sclerotized portion of female genital system.
39. *Hippodamia tredecimpunctata* (L.), dorsal view illustrating numbering of elytral spots. (After Chapin, 1946.)
40. Map of the Upper Mississippi Basin.
41. *Coccinella 9-notata* Hbst., dorsal view illustrating numbering of elytral spots. (After Dobzhansky, 1931.)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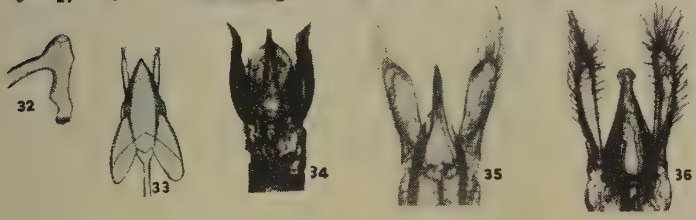
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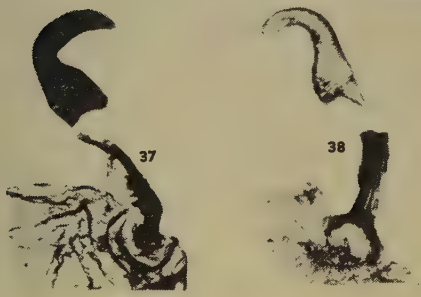
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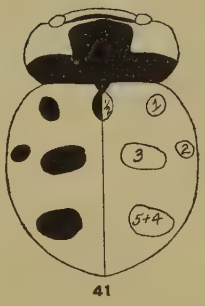
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LITERATURE CITED

1. Blatchley, Willis S.
1910. The Coleoptera of Indiana. Ind. Dept. Geol. Bull. 1, 1,386 pp.
2. Casey, Thomas L.
1899. Revision of the American Coccinellidae. Jour. N. Y. Ent. Soc. 7:71–169.
3. ———.
1905. A new *Carabus* and *Cychrus*, with miscellaneous notes on Coleoptera. Can. Ent. 36:160–164.
4. Chapin, Edward A.
1946. Review of the new world species of *Hippodamia* Dejean (Coleoptera: Coccinellidae). Smithson. Misc. Coll. 106 (11):1–39.
5. Crotch, George R.
1873. Revision of the Coccinellidae of the United States. Trans. Amer. Ent. Soc. 4:363–382.
6. ———. A revision of the coleopterous family Coccinellidae. 311 pp. London, Jansen.
7. Dobzhansky, Th.
1931. The North American beetles of the genus *Coccinella*. Proc. U. S. Nat. Mus. 80 (Art. 14):1–32.
8. ———.
1941. Beetles of the genus *Hyperaspis* inhabiting the United States. Smithson. Misc. Coll. 101(6):1–194.
9. Dodge, H. R.
1938. *Coccidula suturalis* synonymy (Coleoptera:Coccinellidae). Ent. News 49:221–222.
10. Fall, Henry C.
1901. List of the Coleoptera of Southern California. Occ. Papers Calif. Acad. Sci. 8:1–282.
11. ———.
1907. Coleopterological notes, synonymical and descriptive. Ent. News 18:174–177.
12. Herbst, Johann F.
1797. Natursystem aller bekannten in- und ausländischen Insekten, als eine Fortsetzung der Buffonschen Naturgeschichte. Vol. 7, 346, pp. Berlin, Pauli.
13. Horn, George H.
1895. Studies in Coccinelliadae. Trans. Amer. Ent. Soc. 22:81–114.
14. Korschefsky, R.
1931. Coccinellidae I. In Junk-Schenkling. Coleopterorum Catalogus, pars 118, pp. 224. Berlin, Junk.
15. ———.
1932. Coccinellidae II. In Junk-Schenkling. Coleopterorum Catalogus, pars 120, pp. 225–659. Berlin, Junk.
16. Leconte, John Lawrence
1850. General remarks on Coleoptera I. In Agassiz, L. Lake Superior. 4:201–242. Boston.
17. ———.
1852. Remarks upon the Coccinellidae of the United States. Proc. Acad. Nat. Sci. Phila. 6:129–145.
18. Leng, Charles W.
1903a. Notes on the Coccinellidae. Jour. N. Y. Ent. Soc. 11:35–45.
19. ———.
1903b. Notes on the Coccinellidae II. Jour. N. Y. Ent. Soc. 11:139–213.
20. ———.
1908. Notes on the Coccinellidae III. Jour. N. Y. Ent. Soc. 16:33–44.
21. ———.
1911. The species of *Brachyacantha* of North and South America. Bull. Amer. Mus. Nat. Hist. 30 (Art. 12):279–333.
22. ———.
1920. Catalogue of the Coleoptera of America, North of Mexico. 470 pp. Mt. Vernon, N. Y., Sherman.
23. Melsheimer, Friedrich E.
1847. Descriptions of new species of Coleoptera of the United States. Proc. Acad. Nat. Sci. Phila. 3:158–181.

24. MULSANT, ETIENNE
1850. Species des Coléoptères Trimères Sécuripalpes. Annales des Sciences Physiques et Naturelles d'Agriculture de Lyon. Deuxieme Serie. 1,104 pp. Lyon, Barret.
25. NUNENMACHER, F. W.
1911. Studies amongst the Coccinellidae II. Ent. News 22:71–74.
26. PALMER, MIRIAM A.
1911. Some notes on heredity in the Coccinellid genus *Adalia* Mulsant. Ann. Ent. Soc. Amer. 4:283–308.
27. STEHR, WILLIAM H.
1930. The Coccinellidae (Ladybird Beetles) of Minnesota (Coleoptera). Minn. Agr. Exp. Sta. Tech. Bull. 75, 54 pp.
28. ———.
1946. New species of *Scymnus* (Coleoptera: Coccinellidae). Ann. Ent. Soc. Amer. 39:80–81.
29. ———.
1949. Additional notes on *Scymnus lodi* Stehr. (Private communication.)
30. WEISE, JULIUS
1929. Westindische Chrysomeliden and Coccinelliden. Zool. Jahrb. Suppl. 16. 1:11–34.
31. WILSON, J. W.
1927. The male genital tube of some of the species of the genus *Scymnus* (Coleoptera, Fam. Coccinellidae). Psyche 34:167–170.

FISH POPULATION OF A MINING PIT LAKE, MARION COUNTY, IOWA[1]

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Strip mining operations for coal usually leave mounds or hills of sterile, low grade soils turned up on the surface. There were some 2,500 acres of such land in Iowa in 1946 (6). If not rehabilitated, these areas become glaring scars on our landscape. Work has been done by many agencies to reclaim this land for economic purposes. These areas may serve as sites for recreation and wildlife production, and many such sites include pits which frequently may be converted to lakes. Newly abandoned mining pits are frequently very acid, and therefore some of them may not be suitable for fish and other aquatic organisms.

The present study was conducted during the summer of 1950 to determine some of the ecological conditions present in a Marion County pit-lake, usually referred to as the "Ike Lake." It is located 4 miles east of Knoxville, Iowa, just off Iowa Highway 92 (NW¼ of the SW¼ of Section 11, R-19W, T-75N) and has been leased and operated by the Marion County Izaak Walton League from 1944 to the present.

ORIGIN AND DEVELOPMENT OF THE LAKE

The basin in which Ike Lake lies was formed by coal strip mining operations on the long southeast slope of a hill overlooking English Creek. The pit was being actively mined in 1932 and 1933. The overburden was approximately 30 feet thick. The basin left at the completion of operations was on the uphill side of the unleveled waste piles.

In 1945 a road was constructed as an entrance to the area from the highway. The road fill served as a dam at the northeast corner of the lake where any overflow might have taken place, and also formed a diversion ditch for run-off from the field to the north, increasing the watershed by 15 acres. About the same time the mounds of slag and waste surrounding the pit were leveled.

The banks and watershed were planted with various types of cover plants during succeeding years. A black locust, *Robinia pseudoacacia,* shelter was planted along a particularly steep north bank of subsoil in 1496. Red cedar, *Juniperus virginiana;* green ash, *Fraxinus pennsylvanica* var. *lanceolata;* weeping willow, *Salix babylonica;* sandbar willow, *Salix*

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interior; black willow, *Salix nigra;* golden willow, *Salix alba* var. *vitellina;* Mackenzie willow, *Salix mackenzieana;* and Chinese elm, *Ulmus parvifolia,* were planted from time to time with varying success. Plots of reed canary grass, *Phalaris arundinacea,* were established in the mouths of drainage gullies and on some of the steep banks at the shore line. Other vegetation came in voluntarily.

LIMNOLOGY

The surface area of the lake at the time of the study was 4.88 acres. In 1947 the water level was several feet above the 1950 stage. A flat area on the south shore was flooded at that time, increasing the surface area by about 0.5 acre. Due to the abrupt shoreline, a considerable drop in water level would be necessary to appreciably decrease the present surface area.

The shape of the lake is approximately oval in outline (Fig. 1), with few sharp angles in the shoreline. When mapping the lake by the stadia method, it was possible to read all stations from one set-up of the transit.

The watershed is approximately 23.6 acres in area, of which the lake occupies 20.7 per cent. As mentioned above, about 15 acres of the watershed were not available to the lake until the road diverted drainage from the hill to the north. Of the 18.7 acres of land collecting run-off, approximately 15 acres are under cultivation (row and forage crops). A certain amount of erosion from that area is evident at the mouth of the ditch which carries its drainage into the lake. Here an alluvial fan of considerable depth has developed from unloaded silt. The sharply conical shape of the fan indicates that the silt load is dropped at the point where the ditch reaches the lake and spreads out by flowing down the side of the previously accumulated deposit.

The remaining 3.7 acres of the drainage lie on the subsoil and waste resulting from the mining operations. Most of that area has been leveled, and the run-off is relatively gentle except at the point where the drainage falls into the lake.

There has been a certain amount of erosion about the shoreline as a result of wave action. Along the east and north shores the original sides of the pit were sharp and steep. Waves at the water line have cut those banks away and formed a soft shelf of the sediment just below the surface. The inner edge of the shelf falls off sharply into the depths.

According to all indications, surface run-off is the lake's only source of water. No evidence of a source of ground water was found. The water level responded noticeably to a rainfall of one-fourth inch or more (Fig. 2). During the period of study, the lake lost water only by evaporation and seepage. There was no overflow, as the spillway was four feet above the water level. When rains became less frequent, a noticeable daily decline in the water level occurred (Fig. 2).

Seepage was found along the south side, where a dike of waste and slag separates the lake from a deep ditch by approximately 75 feet. At one point, the bottom of the ditch lies about 16 feet below the normal

lake level. Several large seepage spots and one small, steady trickle came from the outside of the dike.

As is characteristic of most pit-lakes, the shoreline drops off sharply, usually to a considerable depth (Fig. 1). The maximum depth is slightly more than 26 feet and is all in one hole approximately 0.5 acre in area. About 75 per cent of the lake is deeper than 10 feet. Along most of the east and north shore, depths of at least 8 feet are found within 10 feet of the water's edge. The volume of the lake on August 1 was approximately

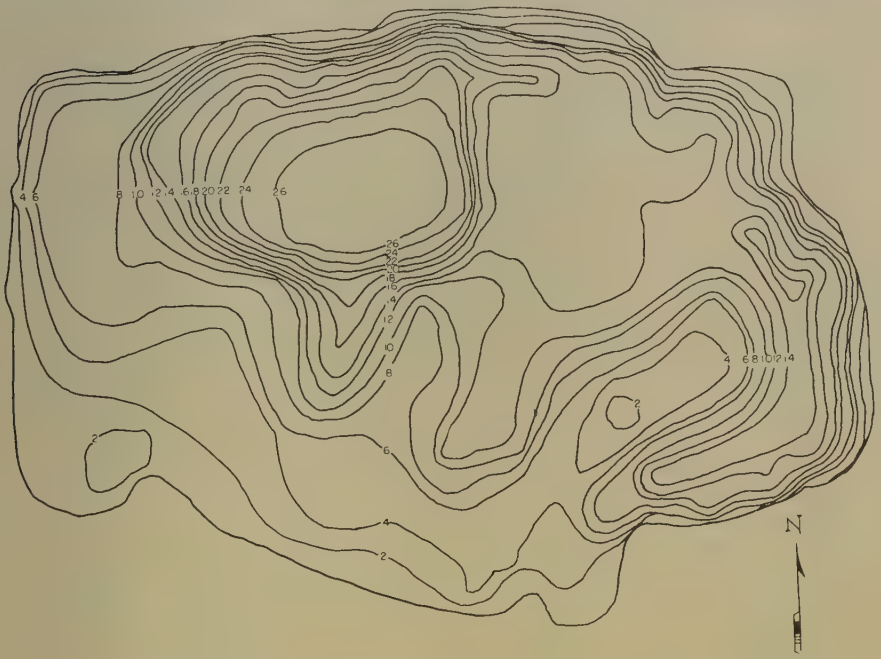

FIG. 1. Map of Ike Lake, Marion County, Iowa, summer, 1950, with two-foot contours marked. Surface area, 4.88 acres.

70.7 acre-feet. That figure was subject to a variation of 3.3 acre-feet between July 10 and September 15.

Determinations of water transparency were taken periodically with a Secchi disk. The disk was lowered on the shaded side of the boat until the disk was lost to sight. Then it was raised to the point at which the observer could discern the black and white disk. This depth is used as an expression of water transparency. A noticeable variation in the transparency of different sections of the lake was detected. The difference was particularly evident on windy days when much "cloudier" water was found on the leeward side of the lake than on the windward side. For

example, the readings of July 22 varied from 39 to 74 inches with a mean of 60. Therefore, the means of four to six readings from scattered sections

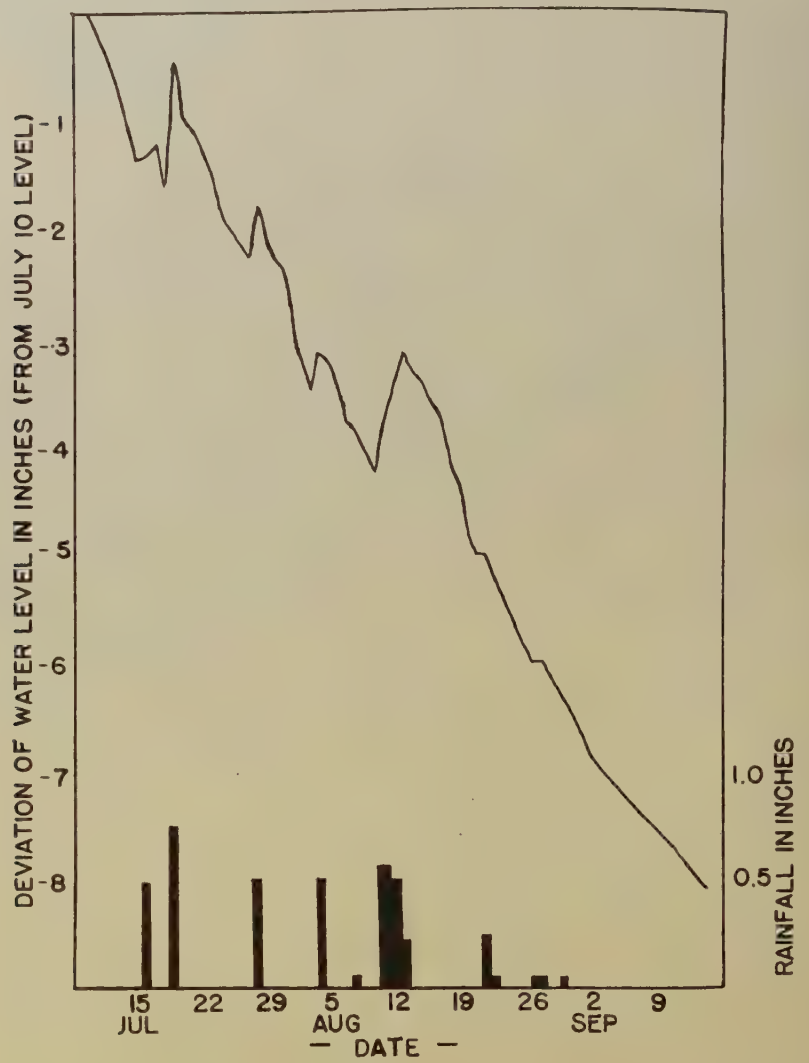

Fig. 2. Rainfall and water level, Ike Lake, 1950.

of the lake were used (Table 1). A trend toward deeper light penetration during the latter part of August was noted.

Attempts were made during July to increase the phytoplankton by

fertilization. On July 6, 160 pounds of an 8-8-8 fertilizer were distributed over the surface and mixed in with an outboard motor. The treatment was repeated using 320 pounds on July 13 and again with 160 pounds on August 1. Two or three days following each application, a slight decrease in transparency could be noted. When it became apparent that a sustained phytoplankton growth could not be obtained with such quantities of fertilizer, the practice was discontinued, and transparency increased. At no time during the study was rain or inflow observed to appreciably increase

TABLE 1
SECCHI DISK READINGS IN INCHES AT IKE LAKE, 1950

Date	Mean Reading	Range	Date	Mean Reading	Range
7–3	50	43–57	7–31	67	58–73
7–6	44	39–48	8–3	44	40–46
7–8	38	36–41	8–7	80	76–86
7–10	51	41–66	8–9	107	80–118
7–12	60	45–70	8–22	84	73–89
7–15	50	47–58	8–28	125	115–133
7–16	43	39–45	9–4	125	116–131
7–22	60	39–74	9–15	81	79–83
7–24	56	49–62			

turbidity in the lake. Roiled bottom soil seemed to have a tendency to settle fairly rapidly.

Temperature records showed the lake to have a thermocline at the beginning of the study on June 28 (Table 2). At that time, the upper and lower limits of the thermocline were found to be at 8 and 16 feet of depth. A general decline took place from July 16 to September 15 when the thermocline lay at 14 to 22 feet. The beginning of a turn-over was indicated during the first two weeks of September by declining surface temperatures and rising bottom temperatures. All temperature records were obtained with a Taylor maximum-minimum weather thermometer.

The dissolved oxygen content was largely governed by the position of the thermocline (Table 3). The epilimnion always contained 7.0 p.p.m. or more of oxygen except immediately adjacent to the thermocline. The oxygen content of the hypolimnion was less than one p.p.m. Hypolimnion conditions were almost completely anaerobic from August 13 to September 15. There was a much sharper drop in dissolved oxygen between the epilimnion and thermocline than between the thermocline and hypolimnion.

The free carbon dioxide content in the epilimnion dropped as the summer progressed and was never greater than 2 p.p.m. after August 6. In the hypolimnion, free carbon dioxide built up steadily during the study. Water samples from the deepest water showed that the free carbon dioxide went from 7.5 p.p.m. on June 28 to 30 p.p.m. on August 28.

Acid titration showed that the lake water contained methyl orange but no phenolphthalein alkalinity. Thus, all alkalinity can be attributed to the bicarbonate ion. Alkalinity in the upper 3 meters ranged from 15 to 23 p.p.m. calcium carbonate and in the next 3 meters from 21 to 44 p.p.m. At the bottom the alkalinity increased during the summer to 141 p.p.m.

Hydrogen ion concentration was, in general, slightly on the alkaline side. The deeper waters tended to be more acid than those at the surface. The range was from *p*H 7.8 at the surface on August 28 to *p*H 6.5 near the bottom on September 15.

AQUATIC PLANTS

The soil and water of the area are apparently reaching a state of fertility more suitable for the establishment of rooted plants. The aquatic vegetation, particularly the submerged plants, formed a rank growth in 1950.

Cattail, *Typha angustifolia,* was the dominant emergent form. The principal stand of cattail was along the west half of the south and southwest shores where the bottom is relatively gently sloping. Along these shores the water depth does not reach 3 feet until 15 to 25 yards from shore. The stand on the north is relatively thin. A heavy growth had become established in a once-submerged draw at the northwest corner and now thrives from 6 to 8 inches above the water line. Several arms of dead cattail stubble extend out into 2 to 3 feet of water from the southwest shore. They were thriving in 1949 until the muskrats cut them. There was no sign of growth in those areas during the present study.

The patches of cattail stubble in the shallow water are apparently attractive to the larger sunfish and bass. During June and July, before the narrow-leafed pond weed, *Potamogeton foliosus,* closed in on the edges, those areas furnished some of the best bass fishing. More rises of bass were noted in that area than in any other. A number of bass nests were located within the stubble.

The submerged vegetation of the pond is made up of narrow-leafed pond weed. In 1950, for the first time, the growth of pondweed became a problem. Most of the water less than 6 feet deep became choked with it by mid-July. Angling and boating in those areas were almost impossible.

During August, it was noted that new beds of pondweed had begun to grow when the transparency of the water had increased from a minimum of 40 inches to a maximum of 133 inches. Deep angling was hindered in places where lines had earlier been free of tangling. Measurements of the water over all known beds showed that growth generally

TABLE 2

WATER TEMPERATURES IN DEGREES CENTIGRADE, IKE LAKE, 1950

Depths in Meters	June	July					August				September	
	28	3	10	16	24	31	6	13	21	28	2	15
Air	.....	26.7	24.4	32.2	22.2	29.4	31.1	20.0	26.7	21.7	21.1	25.0
Surface	24.0	24.4	24.4	26.7	23.3	26.1	26.0	21.7	22.2	21.7	22.2	20.0
1	24.0	24.4	24.4	26.1	22.8	26.7	25.0	20.7	22.2	21.7	22.2	20.0
2	24.0	24.4	24.4	25.0	22.8	26.1	24.4	21.1	21.7	22.2	21.7	20.0
3	20.0	20.0	21.1	22.8	22.2	25.0	23.9	22.2	21.7	22.2	21.7	20.0
4	18.0	16.7	16.7	17.2	17.8	21.7	19.4	20.6	21.1	21.1	21.1	19.4
5	13.0	13.3	14.4	13.9	14.4	15.0	15.0	15.6	17.2	17.2	17.8	17.8
6	12.0	13.9	12.8	12.8	12.8	12.8	12.8	13.3	13.3	14.4	15.0	15.6
7	11.0	12.2	12.1	12.2	12.2	12.2	12.2	11.7	12.8	12.2	12.8	13.3
Bottom	.....	11.1	11.1	.....	12.2	11.7	.....	.....	.....	11.7	.....	.....

TABLE 3

DISSOLVED OXYGEN CONTENT IN PARTS PER MILLION, IKE LAKE, 1950

Depths in Meters	June	July				August				September	
	28	3	16	24	31	6	13	21	28	2	15
1	7.80	7.80	9.20	8.09	7.87	8.58	7.59	8.03	8.25	8.60	8.64
2	.....	.....	8.40	.....	7.03	.....	7.59	7.98	8.03	8.20	8.64
3	.....	.....	.....	.....	7.04	7.59	7.70	6.88	7.04	7.70	7.81
4	.....	0.99	.....	2.04	1.54	1.10	1.10	4.46	3.63	5.89	7.26
5	.....	.....	0.33	.....	1.05	0.20	0.55	0.97	0.55	1.10	1.93
6	.....	.....	.....	0.33	0.94	0.17	0.77	0.97	0.55	0.66	.....
7	0.33	.....	0.33	0.11	.....	.....	0.00	0.00	0.00	0.00	.....

did not reach the surface if it were rooted in water more than 6.5 feet deep. The beds which formed dense mats at the surface grew in water at 5.5 feet or less. The pondweed was not found in water deeper than 8.5 to 9 feet. Areas which were less than 9 feet deep and contained no plant growth were usually found to have an unsuitable foundation. One section of shore was covered with a deposit of coal. Others were soft sediment on which vegetation did not become established.

FISH POPULATION

According to statements of local individuals, black bullheads, *Ameiurus melas,* have been present in the lake at least since 1940. Largemouth black bass, *Micropterus salmoides;* bluegill, *Lepomis macrochirus;* green sunfish, *Lepomis cyanellus;* orange-spotted sunfish, *Lepomis humilis;* and white crappie, *Pomoxis annularis,* have been stocked at various times since 1944 when the lake was first leased to the Izaak Walton League (Table 4). No other species of fish have been reported from the lake. About 25 hybrid bluegill X green sunfish were collected in 1950.

TABLE 4
FISH STOCKING RECORDS, IKE LAKE, MARION COUNTY

Year	Species and Number	Source
1944	40 largemouth bass 40 white crappie 70 bluegills	Fish & Wildlife Service remainder of a load for farm ponds
1945	Mixed (included orange-spotted and green sunfish)	Nearby stream
1945	160 largemouth bass 1,600 bluegills	Fish & Wildlife Service
1947	150 largemouth bass 242 largemouth bass (3–5 inches)	Fish & Wildlife Service Local farm ponds
1948	60 bluegills (3–4 inches)	Local farm pond

Orange-spotted sunfish were taken during an investigation on July 21, 1947, but none was taken during the present study. During the 1947 survey, 4 orange-spotted sunfish were taken to 41 green sunfish. Introduction probably occurred when stockings were made from a local stream.

During the period 1945 through 1948 green sunfish and bullheads are reported to have provided the only fishing. Anglers were first permitted to remove the largemouth bass in 1949. During the summer of 1950 the fishing potentialities of the lake were not utilized as fully as they might have been. There were only about a dozen individuals who fished

in the lake on more than one occasion. A partial creel census was conducted from July 17 to September 4. The fish caught by angling averaged one quarter of a pound. There were 0.85 fish caught per hour of the 85.5 man-hours fished. It is estimated that this census was 90 per cent complete during the period covered. The catch during the 7-week period was apparently a little more than 4 pounds per acre.

POPULATION ESTIMATES

An estimate of the fish population in Ike Lake (Table 5) was made by using the formulae proposed by Schumacher and Eschmeyer (7). The total weights per acre were derived from the population estimates and the average weights of the fish examined. Because of the possible selectivity of the collection methods used, only fish of a total length of 3 inches or larger were marked, and the estimates therefore apply only to fish at least 3 inches long. Data to determine the percentage of the fish which were below 3 inches in total length were not available, but it is felt that those of 3 inches and above comprised a large proportion of the total weight of the fish present.

Marking was accomplished by clipping the left pectoral fin of the scaled fish and the left pelvic fin of the bullheads. Collecting was done largely by means of basket traps. Approximately 90 per cent of the bluegills and green sunfish and 50 per cent of the bullheads were taken in this manner. Seines and hoop nets were also used to a limited extent. All of the bass were caught by angling. Trapping was carried out daily from June 26 to September 1, but angling was intermittent. No predetermined pattern was followed in either method.

During the 10-week period, 52 largemouth bass were marked. These bass were caught by approximately 125 man-hours of angling. On the basis of the recoveries it is estimated, with 95 per cent confidence, that there were between 88 and 201 bass in the lake, or 12.6 to 28.7 pounds per acre.

The estimate for the bluegills, based on 296 marked fish, was 264 to 868 per acre or 9.6 to 31.7 pounds per acre. Most of the bluegills were small, with few over 2 ounces or 5 inches. Since both bass and bluegills reproduced successfully, the pond could be said to be balanced according to Swingle's criteria (1). However, the poundage of bass per acre was almost identical to the poundage of bluegills, thus suggesting an unbalanced condition with too many bass (8).

Green sunfish were definitely not as abundant as the bluegills. The estimate based upon 137 marked fish indicated that there were 33 to 52 per acre or an average of 7.3 pounds per acre. Individuals of over 5 inches in total length made up about 14 per cent of this weight. Apparently the bass and bluegill predation and competition are hindering further successful propagation of the green sunfish.

The black bullheads were all adults with few under 6 inches in total length. It was estimated that there were 31 to 134 bullheads per acre with an average of 17.9 pounds per acre — or almost as many pounds as either

the bass or bluegills. The bullhead estimate was based upon recoveries from only 71 marked fish and is probably the least trustworthy of all the estimates.

FISH MOVEMENT

During the trapping and marking operations, the bass, bluegills, and green sunfish over 5 inches in total length were supplied with numbered

TABLE 5
POPULATION AND STANDING CROP ESTIMATES FOR IKE LAKE, 1950

	Bass	Bluegill	Green Sunfish	Bullhead	Total
Fish marked (Sept. 1)	52	296	137	71	556
Total population					
Estimate	145	2,762	207	402	3,516
Standard Error	27.7	736.5	23.0	124.7	.....
Confidence Limits:*					
Lower	88	1,286	161	151	1,686
Upper	201	4,237	253	653	5,344
Population per acre					
Estimate	30	566	42	82	720
Confidence Limits:*					
Lower	18	264	33	31	345
Upper	41	868	52	134	1,095
Standing crop (lbs/acre)					
Estimate	20.7	20.7	7.3	17.9	112.9
Confidence Limits:*					
Lower	12.6	9.6	5.7	6.7	54.2
Upper	28.7	31.7	8.9	29.0	171.6

* C. L. = mean $\pm$ $t_{.05}$ (standard error of mean).

jaw tags in addition to being fin clipped. That included all bass, yearling or older, and bluegills and green sunfish 3 years old or older.

Since the movement study was only an incidental part of the problem, no fixed trapping pattern was established. This fact and several other factors to be discussed later rendered the data impractical for application of the Chi-square method of statistical analysis. The small

number of fish tagged and consequent smaller number of recaptures also tend to make the data inconclusive.

During the 9-week period, tags were placed on 50 bass, 20 green sunfish, and 2 bluegills. Thirteen bass and 16 green sunfish were recovered one or more times.

The bass were recaptured as little as 30 feet from the original point of marking and as much as 500 feet (Table 6). The greatest linear movement that would have been possible within the lake was 650 feet. The collection of accurate data on bass movement in such a small body of water would probably necessitate a high marking and recovery rate per acre. Individuals would have to be recovered at least three times to give any suggestion of a territory. It must also be recognized that angling is quite selective in its sampling. Anglers generally do not utilize the entire lake. Some areas, particularly the center of the lake, are fished very

TABLE 6

Linear Distances Between Points of Marking and Recapture of Ike Lake Bass, 1950

Distance (Feet)	Number of Fish
Less than 100	2
100–199	3
200–299	4
300–399	1
400–500	3

little. Therefore, data collected by this method would not necessarily show complete patterns of movement. It is felt that accurate conclusions cannot be made concerning tendencies toward either restricted or unrestricted movement. It was conclusively demonstrated, however, that some individuals moved the full length of the lake, 500 feet, in a matter of days.

The green sunfish was somewhat more susceptible to recapture, and individuals were taken as many as six times. This provided better data on their movement patterns. Fish were taken by both angling and trapping. All sunfish were released at one point on the south shore of the lake. Under this plan, if a fish returned to the same general area of his capture, his homing tendency was more strongly demonstrated than if he were released over the trap in which he was caught. Two recaptures could therefore be assumed at least to suggest a tendency of the individual to prefer one area of the lake. Records were obtained on 9 green sunfish which were recaptured two to six times. Seven of these individuals were recaptured from two to four times within territories of 150 feet diameter. Three of these territories are based upon three or four recaptures.

The data suggested that while some individuals were restricted in their movements, others moved about widely. Two individuals were taken at least once in every quarter of the lake. One individual was taken twice in the same day in traps 300 feet apart. It is not known if such erratic movement can be attributed to factors such as age or sex. It was noted that cases of wandering from territories which were fixed by two to four recaptures seemed to occur during the latter part of the summer. Although the evidence of such seasonal movement is by no means conclusive, it sugests that the territorial tendency may be present during only part of the year.

GROWTH STUDIES

Largemouth Black Bass

The largemouth black bass has been recognized as probably the most suitable predatory species to be introduced into small artificial impoundments in the Midwest. In Ike Lake the major part of the man-hours spent at angling was directed at the bass. About 42 bass were taken by angling, other than the investigator's efforts, between June 28 and September 1, 1950.

Since most of the larger bass examined during the investigation were returned to the water for population and movement studies, data on stomach contents of only 12 bass of one year or older were secured. Of those, four stomachs were empty. Aquatic insects were found in six. Three stomachs contained fish, viz: a 2.8–inch bass, a 2.4–inch green sunfish, and a 2–inch bluegill.

Stomach contents of 34 young-of-the-year bass ranging in size from 2.1 to 4.7 inches were examined. Eight stomachs were empty. Among 9 fish examined between July 25 and August 2, there were five occurrences of plankton, mostly microcrustaceans, and two occurrences of insects. Between August 9 and August 23 plankton disappeared from the diet. In 11 fish there were seven occurrences of insects and two of fish. A sunfish 0.9 inch long was found in a 2.6-inch bass, and a 3.4-inch fish contained fish remains. Plankton again became the chief item in the diet when there were eleven occurrences of plankton among 14 fish examined between August 24 and 31. There was only one occurrence of insects. It might be significant to note that a heavy population of mayflies was present on the area up to about August 25. Zooplankton was known to be fairly stable in density throughout the period. From these observations one might detect a definite preference of young-of-the-year bass for insects over zooplankton.

Examinations of 52 bass revealed a high incidence of the white grub of the liver, *Posthodiplostomum minimum*, in the metacercaria stage. All 41 of the young-of-the-year bass were infected. Infestation seemed to be centered around the liver, but quite often surrounding organs, including the spleen, kidney, and gonads, were heavily infected. The livers of a number of young-of-the-year were a mottled white color rather than the natural red. Three of the larger bass were not infected, and the in-

festation of the other 8 was relatively light. The effect of the parasite upon the population cannot yet be determined. In no case did the actions or external appearance of the bass suggest internal parasitism. No other parasites, external or internal, were observed in the bass.

The spawning of bass during the 1950 season was quite heavy. It is very likely that the natural reproduction was the heaviest that has occurred in the lake. Young-of-the-year bass were more evident during the study than young of all other species. Four abandoned nests were located in cattail stubble in water 12 to 18 inches deep after the spawning was completed. Fingerling bass were first observed in schools on July 22. On that date, six separate schools of 200 to 300 fish each were observed. The fish were 2 to 3 inches long. On July 24 a school of smaller fingerlings (under 2 inches) was observed. On July 25 the first fingerling bass was taken in a basket trap. The capture of young bass became a regular occurrence from that date to the end of the study.

As the summer progressed the fingerlings began to lose their schooling tendencies and were quite well distributed throughout the lake. It was possible to locate at least two or three fingerlings within any 10-foot section of shore at almost any time. It is felt that the 1950 bass reproduction was too heavy for the best interests of the fishery. Unless a heavy mortality occurs among the fingerlings the growth of the year class may be very slow.

The length-weight relationship of all bass, yearling and older, is expressed by the equation:

$$\text{Log } W = -3.717 + 3.330 \text{ Log } L$$

where W = weight in thousandths of a pound
and L = total length in tenths of an inch.

The equation is based upon the observed lengths and weights of 72 bass ranging from 4.6 to 17.6 inches in length. From the measurements of 108 fish it was determined that the standard length was 0.816 of the total length and the fork length was 0.952 of the total length.

The coefficient of condition, C, was calculated on the basis of the total lengths and weights of 61 specimens. The formula used is:

$$C = \frac{W\ (10^5)}{L^3}$$

where W = weight in thousandths of a pound
and L = total length in tenths of an inch.

The C-factors showed a gradual increase with increased total length of the fish (Table 7). The average coefficient of condition for the entire sample is 51.0.

The growth of the bass in Ike Lake can probably be considered about average (Table 8). It appears that 3 years are required to produce a legal-sized bass of 10 inches.

Swingle and Smith (9) state that in studies in Alabama the minimum spawning size was found to be 5 ounces, which corresponds to a total length of 9 inches in this population. The average Ike Lake bass

reaches 9 inches during the third summer, and should spawn during that or the following summer.

Bluegill

The bluegill fills the role of forage fish and of pan fish in the bass-bluegill combination for small artificial lakes and farm ponds. In the present population, the adult bass are not totally dependent upon the bluegills for food since small green sunfish are also present. The bluegill in the lake has yet to prove its fishing qualities to the angler.

Zooplankton mostly microcrustaceans and insects made up 82 per cent of all food occurrences in the 203 stomachs examined. Insects were found in 93 stomachs and zooplankton occurred in 59. There was no

TABLE 7

Coefficients of Condition of Bass From Ike Lake, Summer, 1950

Total Length in Inches	Number of Specimens	Mean C	Total Length in Inches	Number of Specimens	Mean C
6.5– 6.9	3	44.2	11.5–11.9	5	43.0
7.0– 7.4	3	39.9	12.0–12.4	2	43.0
9.0– 9.4	1	39.9	13.0–13.4	1	54.3
9.5– 9.9	5	42.6	15.0–15.4	1	55.8
10.0–10.4	7	43.1	15.5–15.9	2	54.7
10.5–10.9	22	41.9	17.0–17.9	1	63.9
11.0–11.4	16	41.5	17.5–17.9	2	67.1

significant change in the food contents of fish of different sizes and the stomach contents were approximately the same for bluegills from 2.0 to 7.3 inches long. Occurrences of plant material in 6 stomachs, algae in 24, and a snail in another were apparently incidental. There were 43 empty stomachs and 2 others contained digested material that could not be identified.

The white grub of the liver occurred in the bluegill much as it did in the bass — however, it was not quite as common. A sample of 208 fish contained 56 (27 per cent) which were infested in varying degrees. Most of those infested had only one to eight parasites visible in the liver. Less than 10 fish were judged to be heavily parasitized.

In judging the developmental stage of the gonads of the fish, any noticeable swelling was accepted as an indication that the fish would, under normal conditions, spawn or produce milt at some future date during the season. The males with testes showing growth and development ranged from 2.3 to 5.4 inches in length, while the females with developing ovaries were from 3.5 to 7.3 inches long. The ovaries of a 7.3-inch female taken on June 28 weighed 42 grams when removed or 25

per cent of the total body weight. Bluegills that appeared to be ready to spawn within one or two weeks were taken up to the last of July.

On July 27, a minnow seine sample included 23 young-of-the-year bluegills ranging from 0.5 to 1.2 inches in length. A similar sample taken on August 17 produced 8 bluegills ranging from 0.8 to 1.3 inches in total length.

All bluegills taken from June 27 to July 15 were used as a sample for calculation of the length-weight relationship. The group included 214 fish from 1.7 to 7.3 inches in total length. The resulting length-weight formula is:

$$\text{Log } W = -3.304 + 3.156 \text{ Log TL.}$$

Measurements of 214 fish indicated that standard length equals 0.789 total lengths, and fork length equals 0.950 total lengths.

TABLE 8

Calculated and Measured Total Lengths in Inches of 71 Largemouth Black Bass From Ike Lake, Summer, 1950

Age Class	Number of Specimens	Average Calculated Length at Each Annulus					Length at Capture
		1	2	3	4	5	
I	7	4.17					6.61
II	10	3.61	8.77				10.07
III	50	2.82	6.81	10.32			11.21
V	4	2.13	5.50	8.80	13.15	16.40	16.90
Mean total length		3.03	7.03	10.21	13.15	16.40	10.92
Mean annual increment		3.03	4.22	3.38	4.35	3.25	
Calculated weight in pounds based upon mean total length*		0.008	0.127	0.440	1.02	2.18	

* $\text{Log } W = -3.177 + 3.330 \text{ Log } L$

An attempt was made to correlate the coefficients of condition of the bluegill with the progression of the season, but no consistent increase or decrease in C-factor could be detected. The coefficient does increase, however, as the total length increases (Table 9). It will be noted that each of the high coefficients for the two larger classes is based upon only one individual. Because of possible errors in weighing small fish, only fish with a total length of 3 inches or more have been used in the calculation of condition coefficients. The coefficient of condition, 64.1, of bluegills of the present population compares quite favorably with that of bluegills in other lakes and ponds in southern Iowa (2,3,5).

Scale samples of 308 bluegills ranging from 2.0 to 8.3 inches in total length were used for the calculation of bluegill growth. The numbers of specimens in each size group are probably not representative of the

numbers in the corresponding size groups of the population since all specimens over 4 inches in length were used, whereas only a portion of the smaller fish was examined.

At the present growth rate, at least 5 years are required for the lake to produce 4-ounce bluegills (Table 10). However, when the growth rate of Ike Lake bluegills is compared with that of other populations, the rate appears to be about average.

Green Sunfish

The green sunfish occupies a rather interesting position in the population. According to information gathered from individuals who have been familiar with the lake for some time, the sunfish was once predominant in numbers. Since the introduction of bass and bluegills, however, the population has dropped considerably. If the samples taken

TABLE 9
COEFFICIENTS OF CONDITION OF BLUEGILLS FROM IKE LAKE, SUMMER, 1950

Total Length in Inches	Number of Specimens	Mean C
3.0–3.4	212	61.0
3.5–3.9	98	63.5
4.0–4.4	37	67.8
4.5–4.9	28	70.6
5.0–5.4	25	72.9
5.5–5.9	1	72.4
6.0–6.4	1	71.3
7.0–7.4	1	96.4
8.0–8.4	1	91.9

during the study are an indication of the relative abundance, the bluegill is now two to three times as numerous as the green sunfish.

Unlike the green sunfish of many other populations in this region, the green sunfish in Ike Lake have produced a number of quite large fish. The individuals of this larger size, as well as some voracious smaller greens, provided the fisherman with far more angling than did the bluegill. It also is felt that these larger greens are filling a second role as a predator as well as forage species.

During the study 67 green sunfish stomachs were examined to determine the general diet. It must be pointed out that most of the green sunfish examined were taken in basket traps. The traps were lifted every 12 hours, and it is possible that fish taken early in the 12-hour period had digested all or part of the stomach contents by the time they were examined. It is also likely that many ate smaller fish held in the trap.

In general, insects of various families and fish made up the major portion of the green sunfish diet. Of 49 fish which were less than 4.5 inches in total length, 27 contained insects. Plant material found in 2 represented the only other food items. Of the 49 stomachs, 22 were empty. In the 19 larger fish examined, insect remains occurred six times and fish ten times. One large fish contained two snails, and five stomachs were empty. The smallest green sunfish found to prey on fish was 4.8 inches in total length. It contained a 2.2-inch bluegill. All of the fish taken by green sunfish were bluegills and ranged from fry to 2.5 inches in length.

The only parasite found in green sunfish was the white grub of the liver, *Posthodiplostomum minimum*. Of 71 fish examined, 32 were para-

TABLE 10

CALCULATED AND MEASURED TOTAL LENGTHS OF 308 BLUEGILLS FROM IKE LAKE, SUMMER, 1950

Age Class	Number of Specimens	Average Calculated Length at Each Annulus in Inches				Length at Capture
		1	2	3	4	
I	183	1.28				2.73
II	87	1.58	3.29			4.10
III	36	1.78	3.10	4.18		5.09
IV	2	1.45	2.95	4.55	6.40	7.80
Mean total length in inches		1.43	3.23	4.20	6.40	3.43
Mean annual increment		1.43	1.59	1.11	1.85	
Calculated weight in pounds based upon mean total length*		.002	.016	.043	.139	

* Calculated from length-weight equation: Log W = −3.304 + 3.156 Log L.

sitized. Although the incidence was nearly 50 per cent, the infestation of individuals was considerably lighter than in the bass. Only one, a 3.2-inch fish, was heavily infested.

Little data concerning the reproduction of the green sunfish were obtained during the study. In a sample of 64 green sunfish examined for condition of the gonads, the sexes were equally divided. Sixteen fish, again at a one to one sex ratio, were at a condition in which the gonads had noticeably enlarged, and ovules were visible in the ovaries. Green sunfish which showed such a condition were taken only between July 7 and August 5. In this stage of development there were 2, 12, and 2 of the I, II, and III-age classes respectively. They were from 3.6 to 5.8 inches in total length.

Less than 20 young-of-the-year green sunfish were seen in two series of hauls with the minnow seine taken on July 27 and August 17.

The length-weight relationship of the green sunfish can be expressed by the equation:

$$\text{Log } W = -3.314 + 3.290 \text{ Log TL.}$$

The observed lengths and weights of 290 green sunfish, ranging from 2.1 to 7.5 inches in total length, were used to compute the equation. The standard length is equal to 0.806 total lengths, and fork length equals 0.966 total lengths.

The mean coefficient of condition of the green sunfish, computed from the total lengths and weights of 195 fish, was 71.7. The 175 specimens, 3.0 to 5.4 inches long, had an average coefficient of 70.9. The 20 fish from 5.5 to 7.5 inches long had a coefficient of condition of 78.7.

The oldest green sunfish taken during the course of the study were 3 years old (Table 11). The growth rate of those green sunfish, while good, was not unusual.

Black Bullhead

Many fishermen prefer to fish for bullheads rather than the other species present. The bullheads are believed to have been present since 1940, but the exact date and circumstances surrounding their stocking are unknown. The fishing success in recent years has been fair to poor. During the study a few bullheads were taken by anglers in water 10 to 15 feet deep.

The bullhead population may be closely controlled by the predation on its eggs and young. No small bullheads were seen during the investigation. The smallest bullhead taken was 6.1 inches in total length. Bullheads may some day be greatly reduced in numbers or even disappear from the lake as the bass increase in number.

The stomachs of 31 bullheads were examined for an indication of their feeding habits. A large part of the contents was made up of fish. There were nine such occurrences. It is felt, however, that the occurrence of fish does not represent the natural feeding habits, since there were indications that the bullheads took fish from the traps in which they, themselves, were caught. They demonstrated a ravenous appetite for small bluegills when they could get them. One 7.8-inch bullhead contained five bluegills which were from 2 to 3 inches in total length. On a number of occasions bullheads regurgitated entire bluegills when handled, indicating recent feeding. Other food occurrences included three instances of insects and a ball of filamentous algae. Of the 31 examined, 20 stomachs were empty.

Infestations of *Posthodiplostomum minimum* occurred in 9 of the 31 bullheads examined. The liver and kidney were the only organs noted to be affected, and all infestations were light. Each of the 130 bullheads handled during the study was examined for external parasites. Leeches were found on the fins of 7 of them. As many as four leeches were found on one fish.

The length-weight relationship of the bullheads is expressed by the equation:

$$\text{Log } W = -3.272 + 2.977 \text{ Log } L$$

That equation is based upon the observed total lengths and weights of 128 bullheads ranging from 6.1 to 9.7 inches in length. Since smaller fish were not available for inclusion, that equation may not be satisfactory for all bullheads that might appear in the lake. Standard length equals 0.821 total lengths.

The coefficients of condition for 128 bullheads were calculated using weights and total lengths ranging from 6.1 to 9.7 inches. Since the C-

TABLE 11

CALCULATED AND MEASURED TOTAL LENGTHS OF 289 GREEN SUNFISH FROM IKE LAKE, SUMMER, 1950

Age Class	Number of Specimens	Average Calculated Length at Each Annulus in Inches			Length at Capture
		1	2	3	
I	226	1.38			3.11
II	49	0.97	3.24		4.89
III	14	1.61	3.41	5.67	6.47
Mean total length in inches		1.32	3.28	5.67	3.57
Mean annual increment		1.32	2.16	2.26	
Summation of annual increments		1.32	3.48	5.74	
Calculated weight in pounds based upon mean total length		0.001	0.024	0.146	

factors for half-inch groups show no consistent trends, the average coefficient of 51.2 was calculated for the entire sample. A comparison of the present population with other populations in Iowa shows that Ike Lake bullheads are in comparatively good condition (2, 3, 5).

Vertebrae for age determination were taken from 20 specimens, and the method described by Lewis (4) was used in aging those fish. Three specimens were discarded because their vertebrae did not show distinct annuli. The remaining 17 specimens proved to be 3 years old. They ranged from 6.6 to 7.9 inches in total length with a mean of 7.3 inches.

The lack of older or younger fish in the sample is significant. It may be interesting to note that all of the bullheads were spawned in 1947 when the present dominant year class of bass was stocked. Swingle and Smith (9) state that the yellow bullhead, *Ameiurus natalis*, eventually disappears from ponds containing largemouth bass.

SUMMARY

1. The present study was conducted during the summer of 1950 on Ike Lake, which was formed in an abandoned coal mining pit near Knox-

ville, Marion County, Iowa. The local Izaak Walton League leased the area in 1944 and developed it as a club grounds.

2. The lake was 4.88 acres in surface area and had a maximum of 26 feet of depth during the study. There are 23.6 acres in the watershed, of which 15 acres are cultivated.

3. During the summer of 1950 more water was lost by evaporation and seepage through the south dike than was gained by run-off, the only known water source.

4. A thermocline was present in the lake and ranged from between 8 and 16 feet to 14 and 22 feet. Dissolved oxygen was always 7 p.p.m. or more in the epilimnion and less than one p.p.m. in the hypolimnion. Free carbon dioxide was never greater than 2 p.p.m. in the epilimnion and built up to 20 p.p.m. in the hypolimnion. All alkalinity was due to the bicarbonate ion, and, in general, the *p*H ranged from 6.5 near the bottom to 7.8 at the surface. Secchi disk readings ranged from 38 inches on July 8 to 125 inches on September 4.

5. The cattail and narrow-leafed pond weed were the only forms of aquatic vegetation present. The pond weed formed a dense growth in water less than 6 feet deep, and when the transparency of the lake increased during August, new growth appeared in water as deep as 8.5 to 9 feet.

6. The fish population included six species. Largemouth black bass were stocked in 1944, 1945, and 1947. Bluegills were established in 1944 and were stocked again in 1945 and 1947. Green sunfish and orange-spotted sunfish were introduced from a local stream in 1945, but none of the latter was taken during the study. Bullheads were brought in sometime before 1940.

7. Population estimates of four species were made by the marking and recovery technique. There were estimated to be between 88 and 201 bass in the lake or 12.6 to 28.7 pounds per acre; 1,286 to 4,237 bluegills or 9.6 to 31.7 pounds per acre; 161 to 253 green sunfish or 5.7 to 8.9 pounds per acre; and 151 to 653 bullheads or 6.7 to 29.0 pounds per acre.

8. Movement of bass and green sunfish was traced by tagging individuals. Bass appeared to move freely over most of the lake. Seven out of 9 green sunfish appeared to have territories during part of the summer.

9. The adult bass fed mainly on insects, and small fish and young-of-the-year bass seemed to prefer insects and zooplankton. Small bass were heavily parasitized by the white grub of the liver, whereas adults were only lightly infected. There was heavy production of young bass in 1950. Age group III bass between 9 and 12 inches made up the next largest size group. The length-weight formula for yearling and older bass was $\text{Log } W = -3.717 + 3.330 \text{ Log } L$. The mean coefficient of condition was 51.0.

10. Microcrustaceans and insects formed the main diet of the bluegills.

The infestation of white grub of the liver was moderate. The length-weight equation was Log W = —3.304 + 3.156 Log L, and the mean C-factor was 64.1. At least 5 years are required to produce 4-ounce bluegills.

11. Green sunfish fed mainly on fish and insects. About half of the green sunfish contained white grubs of the liver, but infestations were light. According to all indications, the number of green sunfish is diminishing. The length-weight equation was Log W = —3.314 + 3.290 Log L. The mean coefficient of condition was 71.7. Five-inch green sunfish are produced in 3 years.

12. About 30 per cent of the black bullheads contained white grubs of the liver. Apparently there is no successful reproduction of bullheads — all those taken were between 6.1 and 9.7 inches in total length. In this population Log W = —3.272 + 2.977 Log L, and the mean C-factor is 51.2. All of the bullheads were found to be 3 years old.

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LITERATURE CITED

1. Anderson, Wallace L.
1948. Results of an analysis of farm ponds in the Midwest. Prog. Fish. Cult. 10:111–116.

2. Carlander, Kenneth C., and Reynold A. Fredin
1948. Project No. 37. Management of small ponds for fish production. Iowa Coop. Wildlife. Res. Unit Quarterly Rept. Oct., Nov., and Dec., 1948:101–105.

3. ———, and John Parsons
1949. Project No. 39. Yellow pikeperch management. Iowa Coop. Wildlife Res. Unit Quarterly Rept. Apr., May, and June, 1949:49–52.

4. Lewis, W. M.
1949. The use of vertebrae as indicators of the age of the northern black bullhead, *Ameiurus m. melas* (Rafinesque). Iowa State Coll. Jour. Sci. 23:209–218.

5. ———
1950. Fisheries investigations on two artificial lakes in southern Iowa. II. Fish populations. Iowa State Coll. Jour. Sci. 24:287–324.

6. LIMSTROM, G. A.
1948. Extent, character, and forestation possibilities of land stripped for coal in the central states. Central States Forest Exp. Sta., Columbus, Ohio, Tech. Paper No. 109. 79 pp.

7. SCHUMACHER, F. X., AND R. W. ESCHMEYER
1943. The estimate of fish populations in lakes or ponds. Jour. Tenn. Acad. Sci. 18(3):228–249.

8. SWINGLE, H. S.
1950. Relationships and dynamics of balanced and unbalanced fish populations. Ala. Agr. Exp. Sta. Bull. No. 274. 74 pp.

9. ———, AND E. V. SMITH
1943. Factors affecting the reproduction of bluegill bream and largemouth black bass in ponds. Ala. Agr. Exp. Sta. Circ. No. 87, 8 pp.

10. ———, AND ———
1947. Management of farm fish ponds. Ala. Agr. Exp. Sta. Bull. No. 254. 30 pp. (Revised).

THE NATURE OF THE SPARING PHENOMENON
I. ACTIVITY AND NATURE OF HEMAGGLUTININ-INHIBITOR[1]

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It has been noted (1, 2) that washed duck erythrocytes parasitized with *Plasmodium lophurae* introduced into the blood stream survived longer in chicks previously injected with duck plasma than in chicks injected with salt water or uninjected. Later (3), it was learned that more washed parasitized chick erythrocytes survived in the blood stream of ducklings previously injected with chicken plasma than in uninjected ducklings, but the high mortality of parasitized cells in both the test and control series rendered such studies unsatisfactory. The activity of injected duck plasma in behalf of the foreign cells in the blood stream of chicks was called "the sparing phenomenon" (2). (The plasmas of ducks recovered from *P. lophurae* infection and, to a limited degree, non-infected ducks, contain also protective substances which tend to suppress the parasitemia in receptors of such plasmas. The protective function becomes evident after the sparing function has manifested itself.)

Further study (4) showed that the bulk of the sparing substance was in the "pseudoglobulin" fraction and the bulk of the protective factor in the "euglobulin" fraction of the duck plasma, although there was evidence of the presence of some sparing substance in the "euglobulin" fraction and of some of the protective factor in the "pseudoglobulin" fraction. Finally (5), the hemagglutinin-inhibiting property of duck plasma in agglutination reactions involving duck erythrocytes and chick plasma was demonstrated *in vitro*. In the present paper are reported further observations on the activity of the hemagglutinin-inhibiting factor, and new information concerning the chemical nature of the substance in the plasma of the duck which is responsible for its hemagglutinin-inhibiting activity.

MATERIALS AND METHODS

The source of the hemagglutinin-containing plasma was the citrated blood of New Hampshire Red chickens from 6–9 months of age. The

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donors had recovered from lophurae malaria and had not been immunized to duck cells. The antigen was, as before (5), the thrice washed erythrocytes of White Pekin ducks. The red cell suspension employed was a 1:50 dilution of the packed red cells after 5 minutes in the centrifuge at 2,400 r.p.m. instead of the previously used 1:8 dilution of the original red cell concentration in the blood of the duck. The agglutinating system consisted of 0.2 cc. of chicken serum, 0.2 cc. of duck erythrocyte suspension, 0.1 cc. of dilution of the material being tested for hemagglutinin-inhibition, and 0.5 cc. of physiological salt solution, at room temperatures. The erythrocytes were always added some time after the mixture of the other components had been shaken vigorously. Recordings were made of the relative strength of the reactions at the time when the greatest differences were apparent between control and various test mixtures.

An extract was made of the plasma and serum of ducks recovered from lophurae malaria and normal ducks by a method for which we are indebted to Rimington (6). One hundred cc. of duck plasma or serum were diluted with an equal amount of 0.85 per cent NaCl solution, and the *p*H adjusted (electrometrically) to exactly 4.7 with 0.1 N HCl. Steam (generated in a suction flask) was bubbled through the mixture until the temperature reached 82° C., and then for 7 minutes more in order to coagulate the globulins and albumins. The maximum temperature attained was 94° C. After cooling, the clear yellow filtrate was collected by filtering through Whatman filter paper No. 1. Reduction in volume was accomplished in partial vacuum at 50°–56° C. The concentrate was thoroughly mixed with 10 volumes of 95 per cent ethyl alcohol, poured into a 2,000 cc. glass cylinder, and left to stand in the refrigerator at 5° C. overnight. A whitish floc settled to the bottom.

The next morning all but about 100 cc. of the supernate was decanted, and the precipitate separated in the centrifuge in 50 cc. round bottom tubes. The supernate was decanted from these tubes without disturbing the packed precipitate in the bottoms. The tubes were turned on end for about 15 minutes to drain. Then cold air was blown into the bottom of the tubes until the precipitate commenced to checker from drying. The precipitate was dissolved in physiological salt solution by prolonged rubbing and stirring, and the volume was equated to the original 100 cc. The filtrate was usually sparkling clear. This preparation will be referred to as duck plasma seromucoid.

Efforts to prepare mucoid extracts of homogenized organs, as liver, spleen, and kidney, were frustrated by the amounts of glycogen that came through, particularly in the liver extract. The most satisfactory method of extracting these organs for hemagglutinin-inhibiting substance was an adaptation of one of Brunius' (7) for the preparation of "source material" containing the so-called "Forssman hapten." One hundred grams of the duck organs were finely ground in an ordinary household meat grinder. To the ground organ was added slowly, with

constant stirring, 500 cc. of acetone c.p. The mixture was poured into a 2,000 cc. flask, and shaken at intervals (in a shaking machine) for a total of 5 or 6 hours during the next 2 days. Then the acetone was filtered off, and the residue dried on the filter paper at room temperature by a stream of air from an electric fan. After the odor of acetone had become very faint the paper with the filtrate was placed for 24 hours in an incubator at 38° C. for further drying. The dried organ was scraped from the paper, crushed in a mortar, mixed with 600 cc. of 80 per cent alcohol for each 100 g. of dried organ, and the latter mixture was shaken intermittently in a mechanical shaker during 2 days. The filtrate was measured, mixed with 1.5 volumes of acetone c.p., and left in the refrigerator at 5° C. overnight. The precipitate was collected and partially dried as described above for duck plasma seromucoid, dissolved in physiological saline, and the volume made up to 100 cc. Preparations by this process will be referred to by such terms as duck liver extract and duck kidney extract.

For general information about mucoids and glycoproteins the authors are indebted to reviews by Meyer (8,9) and papers by other authors cited in his bibliographies.

Although it was not so stated, the chick plasmas used in the previous (5) experiments with hemagglutinin-inhibition by duck plasma were pooled and preserved by freezing in the refrigerator. After it was discovered by Schwink (10) that freezing increased the titer of the isogglutinins in chick plasma, other experiments were planned to test the effect of freezing the plasmas of individual chickens on agglutination of duck erythrocytes. The plasma from each individual bird was divided into two amounts of which one was frozen for 1 or 2 days in the freezing compartment of a refrigerator at 0° to —5° C. and the other kept unfrozen at 1°—4° C.

RESULTS

1. *Effect of freezing on hemagglutination of duck erythrocytes by individual chicken plasmas.* After preliminary experiments had disclosed that freezing could affect hemagglutination with certain chick plasmas when the antigen was duck erythrocytes, and that duck plasma seromucoid and duck organ extracts could affect hemagglutination with fresh or frozen chicken plasma, an experiment was set up to test plasma from each of the 32 chickens 6–12 months of age available in the laboratory. The chickens were a purely random sample. Five or six of the birds were bled each day over a period of 6 days. The results of the experiment appear in Table 1, which compares simultaneous tests with the fresh and frozen plasma of each chicken, and records the results of treating both the fresh and frozen plasma of each chicken with duck plasma seromucoid. The strength of the hemagglutinating reaction is graded 0, ±, +, x—, x, x+, 2x—, 2x, 2x+, etc. in ascending order.

It will be noted that six fresh plasmas (chicks Nos. 18, 25, 26, 27, 28, 32) did not agglutinate duck erythrocytes. All six were from males, although it may not be of significance because 25 of the 32 chickens available were males. Three of the six plasmas (chicks Nos. 26, 27, 28) did not agglutinate after one freezing and thawing, nor did they do so after a second and third freezing a day apart. Two of them (chicks Nos. 18 and 25), however, exhibited marked hemagglutinin properties after the first day-long freezing, while one (chick No. 32) became feebly agglutinating. Of the 26 plasmas which agglutinated when fresh, 10 (chicks Nos. 1, 2, 6, 7, 8, 10, 14, 19, 22, 31) were strengthened by freezing, 13 (chicks Nos. 3, 4, 5, 9, 11, 16, 17, 20, 21, 23, 24, 29, 30) were unaffected while 3 (chicks Nos. 12, 13, 15) were weakened.

To summarize, freezing enhanced hemagglutination in 13 of the 32 chicken plasmas, reduced it in 3, and left 16 unaffected.

2. *Effect of duck plasma seromucoid on hemagglutination of duck erythrocytes.* It has been stated above that dilutions of both fresh and frozen chicken plasmas were tested with both 0.1 cc. of ½-strength and 0.1 cc. of ¼-strength duck plasma seromucoid in the 1.0 cc. agglutinating system, and that the recording was made for the strength producing the most pronounced effect. Three frozen plasmas (chicks Nos. 26, 27, 28) that did not agglutinate duck cells were not affected by seromucoid (*see* Table 1). Of the 29 agglutinating chicken plasmas, which had likewise been frozen once for 24 or 48 hours and thawed, 26 were more or less inhibited by duck plasma seromucoid in a manner reminiscent of the action of duck plasma (5). Sometimes the inhibition was strong, as in chicks Nos. 2, 14, 18, and 25, especially the latter; while in other cases it was weaker, as in chicks Nos. 6, 9, 10, 12, 13, 16, 17, 19, 24, and 32. The frozen plasma of chick No. 8, which agglutinated strongly, was not inhibited by seromucoid after four freezings and thawings at 24-hour intervals. Table 1 does not record the once frozen plasmas of chicks Nos. 4 and 31 as exhibiting reduced hemagglutination after treatment with seromucoid, but three more 24-hour freezings and thawings both increased their agglutinating strength and rendered them slightly inhibitable with seromucoid.

To summarize, duck plasma seromucoid inhibited hemagglutination by 31 of 32 sufficiently frozen chicken plasmas, but one frozen plasma (chicken No. 8) continued unaffected by seromucoid after four freezings and thawings.

The chick plasmas used in the previously reported experiments (5) were pooled from at least two chicks and had been preserved by freezing. The results of the tests for the effect of duck plasma seromucoid on hemagglutination by fresh chicken plasma are recorded in Table 1, where it may be noted that seromucoid apparently did not affect the reaction of 6 fresh plasmas (chicks Nos. 11, 18, 26, 27, 28, and 32), decreased that of 20 (chicks Nos. 3, 5, 6, 7, 9, 10, 12, 13, 14, 15, 16, 17, 20, 21, 22, 23, 24, 29, 30, 31), and actually increased that of 6 (chicks Nos. 1, 2, 4, 8, 19, and 25). Some of these increases were very pronounced as in the case of chicks Nos. 1 and 8.

To summarize, duck plasma seromucoid decreased the intensity of hemagglutination in 20 of 32 fresh and unfrozen plasmas, or 62.5 per cent; did not perceptibly affect it in 6, or 18.75 per cent; and actually increased it significantly in 6, or 18.75 per cent.

3. *Comparison of the behavior of duck plasma seromucoid and duck plasma.* It has been noted (Table 1) that while duck plasma sero-

TABLE 1

RELATIVE HEMAGGLUTINATION STRENGTHS OF 32 CHICKEN PLASMAS ("CONTROLS") WHEN TESTED FRESH AND AFTER FREEZING; AND WHEN TREATED ("TESTS"), BOTH FRESH AND FROZEN, WITH DUCK PLASMA SEROMUCOID

Chick (Sex)	Fresh Plasma		Frozen Plasma		Chick (Sex)	Fresh Plasma		Frozen Plasma	
	Control	Test	Control	Test		Control	Test	Control	Test
1 M....	X	3X	2X	X	17 M...	X+	X	X+	X−
2 M....	±	+	3X	±	18 M	0	0	3X	X−
3 M....	X	±	X	±	19 M...	±	X	X	X−
4 M....	X	2X	X	2X*	20 M...	+	0	+	±
5 ♀....	2X	X	2X	X−	21 M...	2X	X	2X	X
6 ♀....	X	0	2X	X	22 M...	±	0	+	0
7 M....	X	+	2X	X	23 ♀...	X	+	X	±
8 M....	X	2X	3X	3X	24 M...	X	+	X	+
9 ♀....	X	±	X	X−	25 M...	0	X−	3X	0
10 M....	+	0	X	X−	26 M...	0	0	0	0
11 M....	X	X	X	±	27 M...	0	0	0	0
12 ♀....	2X	X	+	±	28 M...	0	0	0	0
13 M....	2X	X	+	±	29 ♀...	X	+	X	±
14 M....	2X	X	3X	X	30 ♀...	X	+	X	±
15 M....	2X	X+	X	±	31 M...	+	±	2X	3X*
16 M....	X	+	X	+	32 M...	0	0	±	0

* Rendered inhibitable with seromucoid after 3 more freezings.

mucoid strengthened hemagglutination by certain unfrozen chicken plasmas, it generally either retarded the reaction to a certain degree or did not affect it. It retarded the reaction of the frozen plasmas in 31 of 32 cases. Could this effect be attributed to a peculiarity of a particular derivative of duck plasma (seromucoid) quite apart from properties of the whole plasma? To answer this question, tests were made with fresh and unfrozen chicken plasmas selected for exhibiting marked

increased sensitivity with seromucoid (chicks Nos. 1, 4, and 8) and markedly reduced sensitivity with seromucoid (chicks Nos. 5, 12, 13, 14, and 21), but substituting ½-strength and ¼-strength duck plasma for the respective dilutions of seromucoid. The duck plasma used was a sample saved from the pool used to make the seromucoid. The responses with the entire plasma were the same as with the seromucoid made from the plasma, i.e., the same selective effects were observed in each case.

4. *Titers of seromucoid from duck plasma and extracts of duck organs as compared with the titer of the plasma.* It was observed in the course of the work that the titers of substances that inhibited hemagglutination varied over a wide range, and depended upon the individual chicken serving as donor of the plasma and the dilution of the chicken plasma. For comparative purposes, when considerable amounts of chick plasma are required, it has been found feasible to pool the frozen plasmas of four or five chickens known to exhibit hemagglutination inhibition with duck plasma or duck ovomucoid. The pooled and frozen plasmas of chickens 1, 3, 6, and 18, for example, when used in 0.1 cc. amounts, proved ideal for determining the comparative titers of duck plasma seromucoid, duck plasma, duck bile mucoid (made like the plasma ovomucoid), duck bile, and extracts of liver, spleen, and kidneys. Each material was pooled from four grown ducks (three males and one female) immune to *P. lophurae*. The titer in each case was the strength of the greatest dilution which produced discernible inhibition of hemagglutination at the time when the most striking differences were apparent, usually 4–8 minutes after the addition of the duck erythrocytes to the system. Since it was not possible to make all the tests with a single set-up, comparisons were made between certain substances each of which, in turn, were compared with certain others. In this way the following titers, which show in a general way the comparative content of the source materials in hemagglutinin-inhibiting substance, were obtained: duck plasma, 1:2560; duck plasma seromucoid, 1:1280; duck bile, 1:160; duck bile mucoid, 1:160; duck liver extract, 1:2560; duck kidney extract, 1:640; duck spleen extract, 1:1280. While no claim is made that the values obtained for the extracts represent authentic assays, it is nevertheless of great interest that the yields obtained in certain cases were so high in comparison with the titer of the plasma itself.

It should be stated that when a number of individual chick plasmas were employed, titers ranging from 1:160 to 1:10240 have been obtained for duck plasma, duck plasma seromucoid, and duck liver extract, depending on the particular chick plasma used and its dilution.

5. *Zone phenomena.* Puzzling zone phenomena were occasionally encountered, of which the following was one of the more complex. The control tubes each contained 0.1 cc. of frozen pooled chicken serum, 0.7 cc. of physiological salt solution, and 0.2 cc. of 1:50 duck erythrocyte dilution, while the remainder of the array consisted of tubes containing

the same mixture with 0.1 cc. of the salt solution substituted with 0.1 cc. of diluted duck liver extract.

The reactions in the control tubes were recorded 2x. Those in the systems with one-two dilutions of duck liver extract from 1:2 to 1:2048 were read as follows: ± (1:2), + (1:4), x— (1:8), x+ (1:16), ± (1:32), x— (1:64), x (1:128), x (1:256), x (1:512), x (1:1024), 2x (1:2048). It is obvious that the titer of the duck liver extract as an inhibitor of hemagglutination was 1:10240, but there were two dilutions of maximum inhibition (1:20 and 1:320) and two dilutions of maximum agglutination (1:160 and 1:20480). Repetition of the array produced the same results.

6. *The immunological nature of the hemagglutinin-inhibiting reaction.* Evidence for the non-adsorbability of the hemagglutinin-inhibiting factor was presented in a previous paper (5). Subsequent efforts to adsorb on duck erythrocytes the factor from duck plasmas, duck plasma seromucoid, and duck organ extracts have similarly met with failure. The only reasonable conclusion to be drawn from such negative results is that the inhibiting principle reacts, directly or indirectly, with an amboceptor (hemagglutinin) innate in the chicken plasma. If so, it seemed desirable to look for a time factor in the reaction, and in this we were successful. There follows a description of one of these tests.

After a few preliminary tests to learn the range of dilutions that were effective, two identical arrays of tubes were set up with 0.1 cc. of hemagglutinating chicken serum and 0.6 cc. of physiological salt solution in each tube. To the first tube of one array was added 0.1 cc. of physiological salt solution; to the second tube 0.1 cc. of duck plasma 1:128 dilution; to the third tube, 0.1 cc. of duck plasma 1:256 dilution; etc. Two hours later identical respective additions were made to the tubes of the second array. Then the antigen (0.2 cc. of 1:50 suspension of duck erythrocytes) was added to each tube of both arrays as soon as possible. It was observed that hemagglutination was inhibited with 1:1280–1:5120 dilutions of the duck plasma in the first array, but only with 1:1280 dilution in the second array. This experiment has been repeated a number of times with other hemagglutinating chicken plasmas, duck erythrocytes, and duck plasmas, always with the result that a lapse of 15 minutes to 24 hours produced a higher hemagglutination titer than a momentary lapse. In addition, it was observed that a 2-hour contact between chicken plasma and duck plasma resulted in a higher agglutination titer than a 30-minute contact, and that even a 24-hour contact resulted in a higher titer than a 2-hour contact. When time intervals of longer than 2 hours were employed the tubes were kept in the refrigerator. Duck plasma seromucoid substituted for duck plasma produced similar results.

The demonstration of a time factor in the exposure of the amboceptor to the hemagglutinin-inhibitor is proof of either a progressive reaction between the two substances, or of one between the hemagglutinin-inhibitor and a substance which governs the amboceptor.

7. *Characterization of the hemagglutinin-inhibiting principle of duck plasma.* It has been stated that the process used in preparing duck plasma seromucoid was one developed by Rimington (6) for separating seromucoid from defibrinated ox blood, and that the process of preparing duck organ extracts was similar to one employed by Brunius (7) for obtaining the so-called "Forssman hapten." These substances, seromucoid and Forssman hapten, sometimes called blood group A substances because they are present in the erythrocytes of type A human blood, are closely related. It is likely that the latter, a hexoseamine perhaps in combination with a lipoid, occurs in the plasma and tissues as a mucoid or protein-hexoseamine, from which the peptide moiety may become separated during the process of preparation (cf. Meyer [9], p. 259). It is likely that in duck plasma seromucoid most of the polysaccharide is still in combination with the peptide, while in duck liver extract most of it is free.

The active components of both duck plasma seromucoid and duck liver extract are relatively thermostable, for both have been kept in test tubes partly immersed in boiling water for 5 minutes after the temperature inside the tubes attained 99° C. without impairing their hemagglutinin-inhibiting titer. After 15 minutes, however, the titer was reduced approximately one-half. Exposures to 56° C. and 65° C. for 20 minutes and 30 minutes did not affect the titer.

Boiling the seromucoid did not produce clouding or precipitate if it was clear to begin with.

Both plasma seromucoid and organ extracts are soluble in water. When dissolved in physiological salt solution the former is sparkling clear, while the latter is definitely opalescent. The organ extracts foam characteristically when shaken.

Duck plasma seromucoid is insoluble in 95 percent ethyl alcohol (see the method of preparation), as are other seromucoids (cf. Meyer [9], p. 252), and are soluble in water after precipitation with alcohol. Duck liver extract is insoluble in acetone, but soluble in a mixture of 4 parts 95 per cent ethyl alcohol and 1 part physiological salt solution. It precipitates from alcoholic solution when acetone is added.

The *p*H of one lot of duck liver extract determined electrometrically was 7.25, while the values for three lots of duck plasma seromucoid were 6.1, 5.7, and 5.7.

The plasma seromucoid was positive to both Molisch test for carbohydrates and Benedict's qualitative test for glucose, but reacted much more strongly in both tests after mixing with an equal part of 2 N HCl and being kept in a covered beaker at the boiling point of water for 1 hour. Evidently the polysaccharide was split from the peptide by hydrolysis. The liver extract was positive to Molisch and Benedict tests.

A number of qualitative tests were made for protein. Both the seromucoid and liver extract were positive to the ninhydrin test. Seromucoid was positive to the sulphosalicylic ring test wherein it was floated over either a 5 per cent aqueous solution of sulphosalicylic acid

or a solution of 5 per cent sulphosalicylic acid in 20 per cent sodium sulphate solution. Some of the cloudiness of mixtures of sulphosalicylic acid solution and seromucoid, however, disappeared on heating to near boiling. Duck liver extract did not lend itself so well to this test on account of its natural opalescence, but there was no doubt of increased cloudiness in the test.

Seromucoid reacted strongly to the nitric acid ring test. Seromucoid gave a yellow, but not an orange, xanthoproteic reaction, while liver extract reacted but very weakly. Both preparations gave positive biuret tests when more than the minimum required $CuSO_4$ solution was added.

When equal parts of seromucoid and saturated aqueous ammonium sulphate solution were mixed there was clouding, and the precipitate could be separated by centrifugation. Further increases in the concentration of ammonium sulphate by the addition of crystals to the supernate produced exceedingly little to no additional clouding. The redissolved and dialyzed precipitate after ammonium sulphate precipitation lost about 50 per cent of its hemagglutinin-inhibition titer, as was determined in tests comparing the original seromucoid with the redissolved precipitate. Whether the loss is to be attributed to failure of all of the active principle to precipitate, denaturation, dialysis, or removal of active contaminants has not been determined.

DISCUSSION

The observation that freezing for 24 hours increased hemagglutination of duck erythrocytes in 13 of 32 chicken plasmas tested, decreased it in 3 plasmas, while apparently not affecting it in 16 others is interesting, even if the cause is unknown, because it points out differences that can develop in the immunological properties of fresh and frozen plasmas or sera. These are in addition to the differences in the innate properties observed among the plasmas of individual birds, as shown in Table 1. Such individual differences are obscured, of course, when pooled frozen plasmas are used, as in our previous experiments (5).

It was observed that certain dilutions of duck plasma, duck plasma seromucoid, and duck organ extracts almost invariably inhibited hemagglutination of duck erythrocytes by sufficiently frozen hemagglutinating chicken plasmas. These substances, in fact, usually inhibited hemagglutination of the duck cells by the fresh chicken plasma also, but in a few cases (6 of 32) they accentuated the reaction. This amphoteric property of duck plasma and the duck plasma and organ preparations, i.e., inhibition of hemagglutination with one chicken's plasma and intensification of hemagglutination with that of another chicken, is frankly not understood. It would seem, however, that the evidence justifies the triple deduction (1) that certain chicken plasmas naturally contain a hemagglutinin-inhibitor, possibly in loose union with the amboceptor, (2) that this inhibitor is destructible with sufficient freezing, and (3) that under certain conditions it can unite with a component of duck plasma

or organs, and in so doing lose its inhibiting properties, releasing the amboceptor that agglutinates the duck erythrocytes. This inhibitor is not identical with the hemagglutinin-inhibitor of duck plasma, because the former is destroyed by either (sufficient) freezing or heating at 56° C. for 30 minutes, while the latter is not affected by either treatment.

The observations on the length of exposure of agglutinating chicken plasma to duck plasma, or duck plasma seromucoid, can be interpreted only as meaning that a chemical reaction goes on progressively and over a considerable period of time between a component of chicken plasma that agglutinates duck erythrocytes and a component of duck plasma or tissues. When the affinities of the amboceptor of chick plasma, or a substance that regulates the amboceptor, are partially satisfied with duck radicals, the amount of hemagglutination is reduced accordingly.

It is apparent by now that it is meant to imply that the component of duck plasma or tissue that produces the phenomenon of hemagglutinin-inhibition is chemically related to the so-called heterogenetic or heterophilic antigens, of which the Forssman hapten is one. Qualitative tests indicate that the substance is present in the organ extracts, and in combination with peptides in the seromucoid. If any doubt remained that our preparations were responsible for the phenomenon of hemagglutinin-inhibition in frozen chicken plasmas or sera, they were dispelled in experiments which demonstrated that when hemagglutination was enhanced by adding the duck plasma or tissue preparations to a fresh plasma it was also enhanced by the whole duck plasma, and when hemagglutination was inhibited with the preparations it was also inhibited with the whole duck plasma.

Landsteiner (11, 12) and Brunius (7) have reviewed their own work and that of others on the role of "blood group A" substance from various sources in hemolysis inhibition with blood group A specific serum, in complement fixation, and inhibition of isoagglutination of A cells. Since then many new observations have been added, but Rimington (6) stated plainly that the physiological significance of the protein-bound carbohydrates in serum is not known. Experiments are being conducted with duck plasma seromucoid and duck liver extract as substitutes for duck plasma in the sparing phenomenon, and there may be some definite conclusions to announce in the near future.

While, as stated, the hemagglutinin-inhibitor in ducks is chemically related to the Forssman hapten, it is not actually that substance. Neither horse seromucoid, horse kidney extract, nor guinea pig kidney extract will produce the hemagglutinin-inhibition characteristic of corresponding duck preparations. The evidence for this statement will be published later.

Except for the literature on blood group A substance and that covered in the discussion in the previous paper (5), the only additional papers that could be located on agglutination-inhibition of human red cells were those of Lubinski (13) and others cited by him, who observed that diluting a hemagglutinating serum with an antagonistic or pre-

viously adsorbed hemagglutinating serum, instead of with saline, diminished the titer of agglutination considerably. The inhibitor was present in different degrees in any human serum, hence was not type specific. Heating at 56° C. for one-half hour did not affect the inhibitor.

CONCLUSIONS

1. Sufficient low-temperature freezing (0°–5° C.) often increases the hemagglutinating potency of chicken plasma (or serum) when duck erythrocytes are used for antigen, but occasionally reduces it.
2. Duck plasma, normal or following recovery from lophurae malaria, inhibits hemagglutination by all but the exceptional frozen chicken plasmas.
3. Duck plasma usually inhibits hemagglutination with fresh chicken plasma also, but in the case of plasmas from certain chickens it strongly enhances hemagglutination (conglutination?).
4. Hemagglutinin-inhibition is explained by a reaction between a component of duck plasma and hemagglutinin in chicken plasmas, because longer periods of contact between the two plasmas produce stronger agglutinations and higher titers than shorter contacts. It is barely possible, of course, that the inhibitor reacts with a substance that regulates the amboceptor instead of with it directly.
5. Enhanced agglutination by certain fresh chicken plasmas with seromucoid is possibly due to union of the seromucoid with a hemagglutinin-inhibiting principle innate in the chicken plasma, the same as is destroyed by freezing. Like the conglutinin phenomenon, it remains to be satisfactorily explained.
6. Duck plasma seromucoid prepared from normal or immune duck plasma and alcoholic extracts of duck organs (liver, spleen, and kidney) chemically related to the Forssman antigen but definitely not identical with it, can, when substituted for duck plasma, assume the role of duck plasma in the hemagglutination reactions described above.
7. The hemagglutinin-inhibiting substances exist in the blood and tissues of the duck as mucoids.

LITERATURE CITED

1. Becker, E. R., C. E. Brodine, A. A. Marousek, and D. A. Byrd
1949. Iowa State College Jour. Sci. 23:323–41.
2. ———, T. M. Schwink, D. Byrd, and R. Conn
1950. Iowa State College Jour. Sci. 24:325–51.
3. ———, and ———
1950. Zeitschr. f. Hyg. 131:614–16.
4. ———, ———, and C. E. Brodine
1951. Jour. Infec. Dis. 89:16–25.
5. ———, ———, and R. M. Prather
1951. Jour. Infec. Dis. 89:95–102.
6. Rimington, C.
1940. Biochem. Jour. 34:931–40.
7. Brunius, F. E.
1936. Chemical Studies on the True Forssman Hapten, etc. Stockholm.

8. Meyer, K.
1938. Symposia on Quantitative Biology. Cold Spring Harbor, L. I. 6:91–102.
9. ———
1945. Advances in Protein Chemistry by Anson, M. L. and Edsall, J. T. New York. Pp. 249–73.
10. Schwink, T. M., and E. R. Becker
1951. Jour. Parasit. 37 (No. 5, Sec. 2):11–12.
11. Landsteiner, K.
1936. Jour. Exper. Med. 63:185–90.
12. ———, and M. W. Chase
1936. Jour. Exper. Med. 63:813–17.
13. Lubinski, H. H.
1942. Jour. Immunol. 44:95–100.

MORPHOLOGICAL FEATURES OF BRACHYLAIMA VIRGINIANUM METACERCARIAE (TREMATODA: BRACHYLAIMATIDAE), AND MIGRATION ROUTE OF CERCARIAE IN THE SECOND INTERMEDIATE HOST

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During recent studies on the life cycle of the brachylaimid trematode, *Postharmostomum helicis*, the examination of land molluscs in the vicinity of Ann Arbor, Michigan, disclosed the presence of numerous metacercariae belonging to another genus of this family (*Brachylaimatidae*).[1] Morphological studies of living and fixed metacercariae, together with feeding experiments to secure adult worms, indicated the species to be *Brachylaima virginianum* Krull. Numerous references to this trematode are found in the literature, the major aspects of the life cycle having been determined by Krull (1935). This paper, supplementing the author's preliminary report (1951c) deals with certain morphological aspects of the metacercariae and the migration route of the cercariae in the second intermediate host.

HISTORICAL REVIEW

Descriptions of brachylaimid trematodes by early workers are often so incomplete that precise identification becomes almost impossible. A review of the literature dealing with *Brachylaima virginianum* emphasizes the necessity for additional life cycle studies on the other species now assigned to the *Brachylaimatinae*.

Lutz (1895) described and figured *Distoma opisthotrias* from the small intestine of the opossum, *Didelphis aurita* in Brazil and suggested that a land mollusc might be involved as the intermediate host. Braun (1899) placed *Distoma opisthotrias* in his newly erected genus *Harmostomum*, while Looss (1899) included it in his new genus *Heterolope*.

[1] According to McIntosh (1950) and others, the generic name should be *Brachylaima*, since this was the spelling used by Dujardin (1843) in his original description. McIntosh also noted that Blanchard's (1847) emendation of the generic name to *Brachylaemus* should be rejected, in keeping with the recent ruling (Opinion 148) of the International Commission of Zoological Nomenclature. The gender of Dujardin's (1843) *Brachylaima* evidently was neuter, since he employed the specific designation *fulvum* which corresponds to the Greek gender of *laima*. Since family names, according to the International Rules are to be constructed by adding *-idae* to the stem (in this case *Brachylaimat*) of the name of the type genus, the family and subfamily names become *Brachylaimatidae* and *Brachylaimatinae* respectively, and the species here dealt with *Brachylaima virginianum* Krull, 1933.

Braun (1901), because of the inadequacy of Lutz's (1895) description, re-characterized the species and included measurements, using preserved specimens from opossums collected in Brazil. When Witenberg (1925) revised the subfamily *Harmostominae* (=*Brachylaimatinae*), he recorded this species as *Harmostomum* (*Harmostomum*) *opisthotrias* (Lutz, 1895) and as synonyms included those forms described by Looss (1899) and Braun (1901). Dickerson in 1930 described a brachylaimid from the small intestine of the opossum, *Didelphis virginiana* collected in Virginia which he named *Harmostomum opisthotrias* var. *virginiana*. Dickerson considered it a distinct variety because of differences in body size and egg size. No attempts at a life cycle study were made by him. Sinitsin (1931), principally on the basis of morphological similarities between adult worms and larval stages found in nature, revised the subfamily *Harmostominae* (=*Brachylaimatinae*) and listed Dickerson's species, together with several others, as synonyms of *Harmostomum migrans* (Dujardin, 1845), a form from European shrews. Sinitsin found metacercariae in naturally-infected *Polygyra thyroides* (=*Mesodon thyroidus*) and adults in *Didelphis virginiana* in Maryland. Sporocysts and cercariae, which he believed to be of the same species, were also described and illustrated. Because Sinitsin's work lacked experimental verification, however, its validity remains questionable.

Chandler (1932) found opossums in the vicinity of Houston, Texas, infected with *Harmostomum opisthotrias* (Lutz, 1895). In a brief historical resumé of the species, he concluded that Lutz's (1895) and Braun's (1901) species were identical and that his specimens were intermediate in size between those described by Dickerson (1930) and the Brazilian forms. Hence. he doubted the validity of Dickerson's species. When Dollfus (1935), however, illustrated and gave measurements of specimens of *Brachylaemus opisthotrias* (Lutz) from Brazil, showing numerous structural variations which may occur in adults of the same species, Chandler (1946) reversed his earlier opinion and concluded that the North American species are not cospecific with Brazilian ones. Meanwhile, Krull (1933b) in a preliminary report, and in 1935 in a fuller account, had published aspects in the life cycle of *Brachylaemus virginiana* (Dickerson), adults of which he recovered from opossums in Maryland. He considered it synonomous with *H. migrans* of Sinitsin and *Harmostomum* (*H.*) *opisthotrias* var. *virginiana* Dickerson, but not with the Brazilian species, *B. opisthotrias* (Lutz). Aware of the rather striking variations which may be shown by one species, Krull considered that only a single species of *Brachylaima* exists in the Maryland area, contrary to his earlier (1933b) opinion when, in a feeding experiment with opossums, he recovered *Brachylaemus spinosulum* (Hofmann, 1899), a parasite frequently found in European hedgehogs. Nonetheless, Krull believed that *B. spinosulum* and *B. virginianum* should be kept as separate species, on the basis of differences in measurements. Krull's experimental studies on the life cycle of *Brachylaima virginianum* definitely established the land snail *Mesodon thyroidus* as the first and second intermediate hosts. Additional experimental definitive hosts reported by

Krull in 1934 included the white rat, cat, dog, and chick. New intermediate hosts noted by him in 1936 were *Helix pomatia, Deroceras laeve,* and an aquatic snail *Pseudosuccinea columella. Helisoma trivolvis* and *Succinea* sp. were also indicated as possibly serving to harbor the metacercarial stage. Reynolds in 1938 found metacercariae structurally similar to those of *B. virginianum* in naturally-infected *Agriolimax agrestis* in Virginia and listed this slug as a possible host. Chandler (1946) found adult *B. virginianum* in Texas armadillos, and agreed with Krull that *B. virginianum* should be retained as a valid name for the species from North American opossums and armadillos, but that it may have to fall in synonymy with one or more of the earlier described European species. Recently, Hand and Voge (1952) added *Ariolimax columbianus* as an additional secondary intermediate host.

Until more definite information becomes available on the European and Brazilian species, as well as related American ones, it seems likely, therefore, that *Brachylaima virginianum* Krull should stand as the valid designation for this parasite.

MATERIALS AND METHODS

Mesodon thyroidus harboring metacercariae of *Brachylaima virginianum* were collected from various localities in the vicinity of Ann Arbor, Michigan during the period 1946-52. A detailed listing of these regions will be found in the first portion of the writer's (1951a) report on the life cycle of *Postharmostomum helicis.* Parasites removed from the snail were examined both in the living state and in stained preparations. Whole mounts fixed in formalin, F.A.A., or Bouin's were stained with Delafield's haematoxylin, Harris's haematoxylin, Mayer's alcoholic cochineal, and Mayer's HCl carmine. Serial sections of metacercariae and snails harboring them were prepared using the same fixatives noted above and stained in Delafield's haematoxylin and Harris's haematoxylin, with eosin, erythrosin, and triosin counterstains.

To secure adult worms, fully developed metacercariae from naturally-infected *M. thyroidus* were force-fed orally to white mice. All mice used in such feeding experiments were laboratory-raised, to preclude any possibility of previous infection. Cercariae from naturally-infected *M. thyroidus* were exposed to laboratory-reared snails of the same species in studies of the migration route of the cercariae into the second intermediate host. Metacercariae recovered from the kidneys of snails infected in this manner, upon subsequent feeding to white mice developed into adult worms, morphologically similar to those described by other workers dealing with this species. The adults in all cases were localized in the posterior region of the small intestine.

For detailed studies of the excretory system of the metacercariae, the use of neutral red as an intra-vitam stain proved helpful. Metacercariae freshly removed from the snail kidney were placed in tap water in small Stender dishes. One drop of 0.5 per cent aqueous neutral red was added, and the material placed under refrigeration overnight. When examined microscopically following this procedure, inter-

nal organs such as the genital glands, digestive tract, and the major excretory vessels are well differentiated from the surrounding parenchyma. Metacercariae treated in this manner and kept under refrigeration yield excellent preparations for the study of morphological details even as long as a week after removal from the snail host.

All drawings and diagrams were made with the aid of the camera lucida or microprojection apparatus.

SNAIL HOSTS

Of 256 *Mesodon thyroidus* examined, 48 were infected with *Brachylaima virginianum* metacercariae; 47 contained metacercariae of *Postharmostomum helicis* (a related brachylaimid) and four snails harbored metacercariae of both these species. Metacercariae of *B. virginianum* are found principally in the renal chamber of the snail, while those of *P. helicis* are restricted to the pericardial cavity. Occasionally, however, metacercariae of the two species may be situated in either of these regions.

Anguispira alternata, the most frequently encountered terrestrial mollusc in the Ann Arbor area, which serves as the primary and secondary intermediate host for *P. helicis,* also harbors metacercariae of *B. virginianum.* It appears, however, that this snail is not a normal host for the latter species, since of 6533 *A. alternata* examined, only 225 harbored *B. virginianum* metacercariae. Moreover, 181 of these snails contained only dead metacercariae. Reference to these dead metacercariae has been made in an earlier paper (Ulmer, 1951a). A great number of additional land molluscs have been examined, some of which appear to serve as additional secondary intermediate hosts for *Brachylaima virginianum* metacercariae. This report, however, is concerned primarily with a discussion of the parasites harbored by *Mesodon thyroidus.* That brachylaimid trematodes are apparently quite indiscriminate in their selection of secondary intermediate hosts has frequently been noted. On the other hand, the specificity of the sporocyst and cercarial generations for a single species of land mollusc appears to be characteristic for this group of trematodes.

EXCRETORY SYSTEM

Despite numerous studies made on *Brachylaima virginianum,* comparatively little has been done on the metacercarial excretory pattern. Conflicting views as to the arrangement of the principal excretory tubes are frequently found. Lutz (1895) observed in the adult worm a somewhat ventrally situated excretory pore which, according to him, forked into two branches, these almost immediately subdividing again. Dickerson (1930) in describing the adult noted, on the other hand, a dorsally situated excretory pore and the major excretory vessels running "...parallel to the intestines to the pharynx where...the branches...apparently end, each as a blind sac, in the region between the ventral sucker and the pharynx..."

Sinitsin (1931) in his revision of the *Harmostominae* (=*Brachy-*

laiminae) placed considerable significance on the position of the descending limbs ("siphons," he termed them) of the primary excretory tubes in relation to the digestive tract. If external to the ceca, an "ectosiphonous" condition prevailed, and when internal to the intestinal crura, the main excretory ducts were considered "entosiphonous." His division of the subfamily into the tribes Ectosiphonea and Entosiphonea on the basis of this arrangement has found little acceptance, however, by present-day investigators. Adam and Leloup (1934) noted the variability in position of these structures associated with fixation of the parasites. My studies of living specimens, too, indicate that a great deal of variation occurs in this respect, associated principally with the flattening of the specimen by cover-slip pressure.

Sinitsin illustrated the excretory system of the adult which he had recovered from *Didelphis viginiana*. I am in agreement with him in the arrangement of the primary excretory tubes but not so as to the arrangement of the secondary collecting tubules. Ramifications of the excretory system beyond the ascending limbs of the primary tubes were not discussed by Krull (1935). My findings in other respects compare favorably, however, with his illustration of a fully-grown metacercaria.

The rather small Y-shaped excretory bladder opens dorsally (Fig. 1). Just behind the posterior testis, emptying into the cornua of the bladder are two primary excretory tubes, each consisting of a descending limb (DL) and an ascending limb (AL). The ascending limb is ciliated and takes its origin at the posterior portion of the body where it is formed by the junction of the secondary collecting tubules (ST). The ascending limbs, intracecally situated, extend anteriorly to the acetabulum, where each turns laterally and continues anteriad, but now extracecally. At the level of the pharynx each describes a ventral loop, loses its ciliated character and then, as the descending loop, runs posteriad to discharge into the bladder.

The secondary collecting tubules are distributed in the following characteristic pattern: an anterior branch runs forward from the posterior of the body to the region of the acetabulum where it subdivides into tertiary tubules supplying the anterior of the body. The remaining secondary collecting tubule on each side of the body divides approximately at the level of the ovary into two tertiary tubules (TT), one of which turns posteriad to supply the posterior body region, the other subdividing at the posterior level of the anterior testis into two quartenary tubules. One of these supplies that portion of the body situated between the acetabulum and genital glands, the other continuing forward towards the acetabulum, where it subdivides into numerous rami. Despite the large number of tubules involved in the excretory pattern of a mature metacercaria and the hundreds of flame cells present, no anastomoses of branches from one side of the body with those of the other side have been observed. The basic arrangement of primary, secondary, tertiary, and quartenary tubes described above may be traced with little difficulty in mature living metacercariae.

REPRODUCTIVE SYSTEM

The genital glands together with their associated ducts, all well developed in mature metacercariae, exhibit considerable variation. Testes and ovary usually are arranged in a more or less triangular fashion in unflattened specimens. Extended worms, however, may show the genital glands arranged in linear series, the ovary situated between the testes. The ovary may occupy a position either to the right or left of the mid-line in ventral aspect. This sexual amphitypy was noted by Sinitsin (1931) who considered it of no taxonomic significance.

The effect of fixation and pressure is of importance in the general appearance of the genital glands, both as to their shape and size. They may appear lobate or smooth in outline in accordance with variations in cover-slip pressure or type of fixative employed. In specimens freshly removed from the snail host, they most frequently are smooth in outline. Inasmuch as the lobate or smooth nature of the gonads is frequently considered an important criterion in the differentiation of species, there apparently is need for some revision of the taxonomic characters now employed.

The appearance of the intestinal crura similarly shows considerable variation. In extended specimens they are nearly straight, running parallel to the sides of the body to the posterior extremity (Fig. 3). In encysted forms they are convoluted (Fig. 4), similar in appearance to the intestinal crura of members of the genus *Postharmostomum*.

My findings concerning details of the reproductive system of the metacercaria (Fig. 2) are in agreement with Krull's, save for the vitellaria, which Krull stated were apparently undeveloped in the metacercaria. Ducts of the vitelline glands are readily seen in living specimens, though indistinct in most stained whole mounts. The vitellaria, however, appear quite plainly in haematoxylin-stained whole mounts as well as in sections (Figs. 8, 9), but are seen with difficulty in carmine-stained whole mounts.

MIGRATION ROUTE OF CERCARIAE

Previous studies on *Brachylaima virginianum* have not dealt with the migration route of cercariae through the second intermediate host. Sinitsin (1931: 811) suggested that the parasites may reach the renal chamber *via* its outside opening in the mantle cavity but made no observations concerning this. Krull (1935) in a series of feeding experiments which proved conclusively that two intermediate hosts are required for the completion of the life cycle, and that the first intermediate host may also serve as the second intermediate host, did not establish the route whereby cercariae reach the kidney.

In an attempt to determine the precise mode of entry into the second molluscan host, laboratory-reared *Mesodon thyroidus* were exposed at varying intervals to cercariae of *Brachylaima virginianum* from naturally-infected snails of the same species. Subsequent feeding of the mature metacercariae produced adults of *B. virginianum*.

Cercariae from sporocysts were removed from naturally-infected snails by crushing the latter in Syracuse watch glasses. Laboratory-reared *Mesodon thyroidus* were then placed in the containers and after 1 hour were removed and isolated. Additional experiments conducted simultaneously involved the exposure of *M. thyroidus* infected some weeks previously in the laboratory with *Postharmostomum helicis* (a related brachylaemid) to secondary infection with *B. virginianum* cercariae. *Postharmostomum helicis,* as noted above, occupies the pericardial chamber of a variety of land molluscs, reaching the heart by means of the ureters, kidney, and renopericardial canal. Periodic examinations of snails so exposed to cercariae of *B. virginianum* were conducted both by gross dissections and serial sections. Cercariae were seen entering the respiratory orifice of the snail, but further knowledge of the course of migration was dependent upon sections. Cross-sections through the heart and kidney region frequently showed cercariae within the ureter (Fig. 15). The renal organ, into which the ureter opens is thus readily accessible to the parasites, which remain there until maturity. The time involved in the migration is relatively short, some reaching the kidney chamber as early as 12 hours after exposure of the snail. Fully-developed encysted metacercariae lie within the renal chamber, apparently free within its lumen (Fig. 16) and not attached to the renal folds as do the young developing metacercariae.

ABBREVIATIONS USED IN PLATES

(All drawings were made with the aid of the camera lucida or microprojection apparatus.)

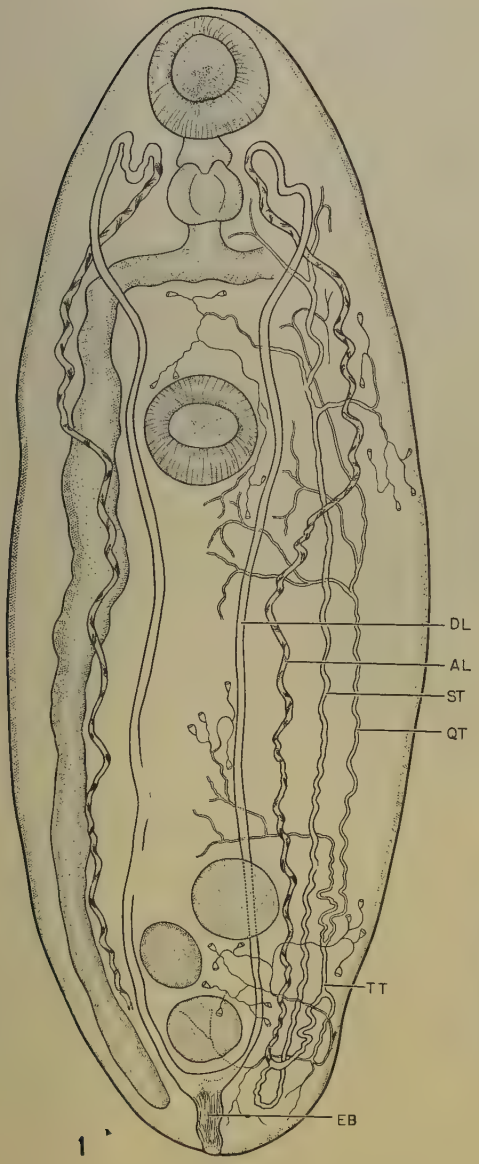
Abbreviations

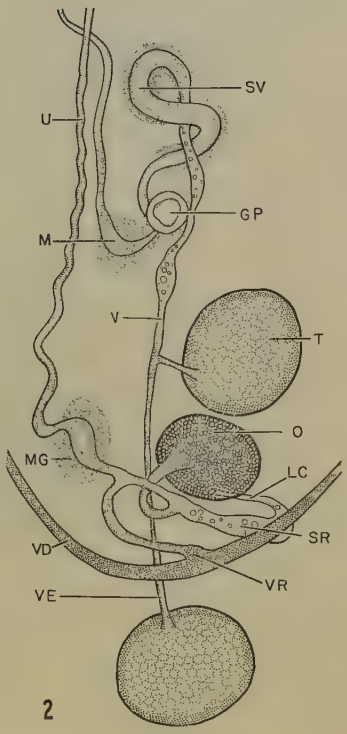
A acetabulum
AL ascending limb of primary excretory tube
DL descending limb of primary excretory tube
EB excretory bladder
GP genital pore
K kidney
LC Laurer's canal
M metraterm
MG Mehlis' gland
O ovary
OS oral sucker
P pharynx
PC pericardial cavity
PU primary ureter
QT quartenary collecting tubule
SR seminal receptacle
ST secondary collecting tubule
SV seminal vesicle
T testis
TT tertiary collecting tubule
U uterus
V vas deferens
VD vitelline duct
VE vas efferens
VR vitelline reservoir

PLATE I

FIG.

1. — Excretory system of mature metacercaria of *Brachylaima virginianum*, ventral aspect. Digestive tract and distribution of flame cells only partially shown. Only major divisions of main excretory ducts shown on left side.
2. — Genital complex of mature metacercaria, ventral view.
3. — Camera lucida drawings of living metacercariae, showing variations in shape and size of body. Note also variations in gut and distance between oral sucker and acetabulum.
4. — Fully developed metacercaria. Note convolutions of gut.





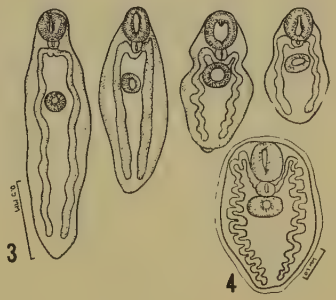


PLATE I

PLATE II

FIG.

5. — Sagittal section through mature metacercaria. Note opening of Laurer's canal.
6–13. — *Series of cross-sections through mature metacercaria.*
6. — Cross-section through the region of the oral sucker.
7. — Cross-section through the region of the pharynx.
8. — Cross-section through the region of the acetabulum.
9. — Cross-section midway between the acetabulum and the genital pore.
10. — Cross-section through the region of the genital pore.
11. — Cross-section through the region of the ovary.
12. — Cross-section through the region of the posterior testis.
13. — Cross-section through the region just anterior to the junction of the primary collecting tubes.
14. — Cross-section through the region of the bladder.
15. — Cross-section through *Mesodon thyroidus* in the region of the heart and kidney. Note metacercariae of *Brachylaima virginianum* in the kidney chamber and primary ureter and metacercariae of *Postharmostomum helicis* within the pericardial cavity.
16. — *Mesodon thyroidus,* sagittal section through kidney region showing metacercariae of *Brachylaima virginianum* within the renal chamber.

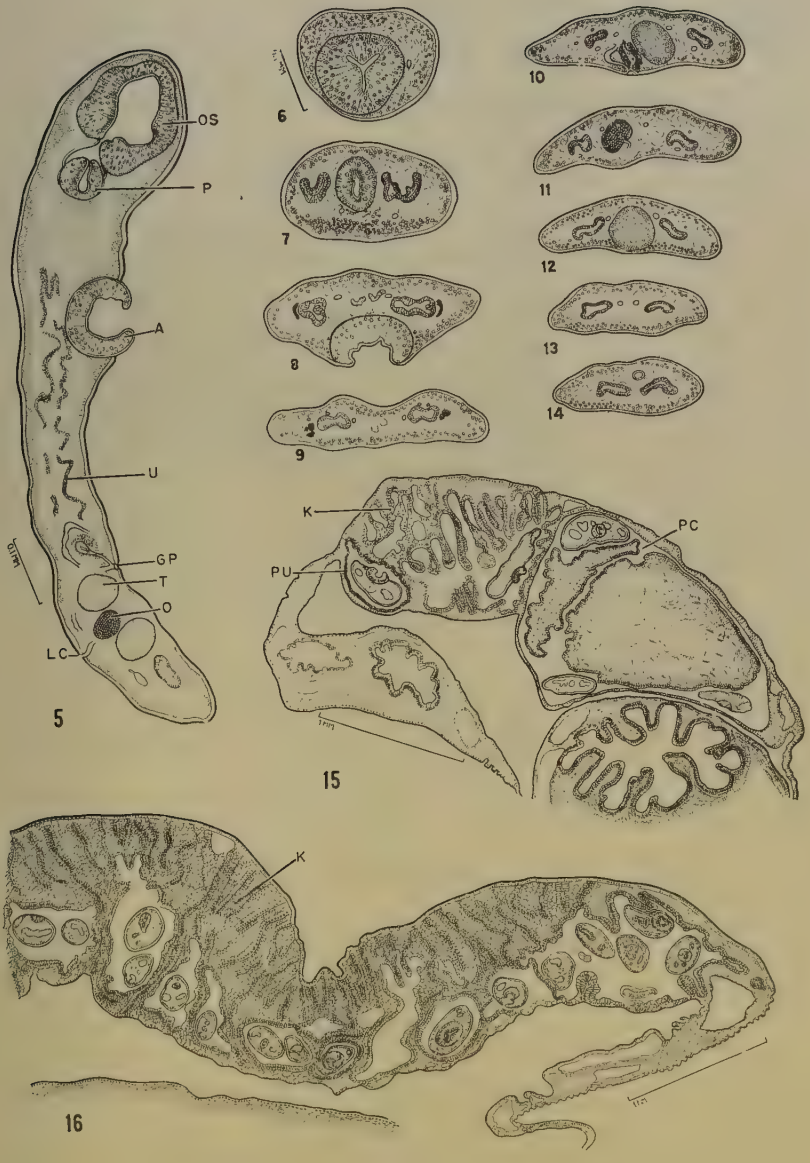


PLATE II

LITERATURE CITED

Braun, M.
1899. Über *Clinostomum* Leidy. Zool. Anz. 22: 489–493.

———
1901. Zur Kenntniss der Trematoden der Säugethiere. Zool. Jahrb., Jena. Abt. Syst. 14: 311–348.

Chandler, A. C.
1932. Notes on the helminth parasites of the opossum (*Didelphis virginiana*) in southeast Texas, with descriptions of four new species. Proc. U.S. Nat. Mus. Vol. 81, Art. 16, 1–15.

———
1946. Helminths of armadillos, *Dasypus novemcinctus,* in eastern Texas. Jour. Parasitol. 32(3): 237–241.

Dickerson, L. M.
1930. A new variety of *Harmostomum opisthotrias* from the North American opossum, *Didelphys virginiana,* with a discussion of its possible bearing on the origin of its host. Parasitol. 22: 37–46.

Dollfus, R. Ph.
1935. Sur quelques *Brachylaemus* de la faune francaise récoltés principalement á Richelieu (Indre-et-Loire). Ann. Parasitol. 13(1): 52–79.

Hand, C. and Voge, M.
1952. *Ariolimax columbianus,* an intermediate host for *Brachylaemus virginiana* (Dickerson) (Trematoda; Digenea) in California. Jour. Parasitol. 38 (1): 57–58.

Joyeux, Ch. and Foley, H.
1930. Les helminthes de *Meriones shawi shawi* Rozet dans le nord de l'Algérie. Bull. Soc. Zool. France. 55:353–374.

Krull. W. H.
1933a. The opossum, *Didelphis virginiana virginiana* Kerr, a new host for *Brachylaemus spinosulum* (Hofmann, 1899). Proc. Helm. Soc. Wash. in: Jour. Parasitol. 20:98.

———
1933b. Studies on the life history of *Brachylaemus* sp. possibly *B. spinosulum* (Hofmann, 1899). Jour. Parasitol. 20: 109–110.

———
1934. New experimental hosts for *Brachylaemus virginiana* (Dickerson) Krull, Jour. Wash. Acad. Sci. 24(11): 483–485.

———
1935. Some observations on the life cycle of *Brachylaemus virginiana* (Dickerson) Krull 1934. Trans. Amer. Micr. Soc. 54(2)118–134.

———
1936. New terrestrial and aquatic intermediate hosts for *Brachylaemus virginiana* (Dickerson) Krull (Trematoda: Brachylaemidae). Proc. Helm. Soc. Wash. 3(2) 56–58.

Looss, A.
1899. Weitere Beiträge zur Kenntniss der Trematoden-Fauna Aegyptens, zugleich Versuch einer natürlichen Gliederung des Genus *Distomum* Retzius. Zool. Jahrb., Jena. Abt. Syst. 12: 521–784.

Lutz, A.
1895. *Distoma ophisthotrias,* um novo parasita do gambá. Rev. Museu Paulista Sao Paulo, 1: 181–193.

McIntosh, A.
1950. *Brachylaima rauschi* n.sp., from an Arctic lemming, *Dicrostonyx groenlandicus rubricatus* (Richardson, 1839). Jour. Parasitol. 36(6):20 (Abstract).

Reynolds, B. D.
1938. Metacercariae of a species of *Brachylaemus,* probably *B. virginianus* from *Agriolimax agrestis.* Jour. Parasitol. 24(4): 377–378.

Sinitsin, D.
1931. Studien über die Phylogenie der Trematoden. V. Revision of Harmostominae in the light of new facts from their morphology and life history. Ztschr. Parasitenk. 3(4): 786–835.

ULMER, M. J.
1951a. *Postharmostomum helicis* (Leidy, 1847) Robinson, 1949, (Trematoda), its life history and a revision of the subfamily Brachylaeminae. Part I. Trans. Amer. Micr. Soc. 70(3):189–238.

———
1951b. *ibid.* Part II. Trans. Amer. Micr. Soc. 70(4): 319–347.

———
1951c. Studies on brachylaemid metacercariae (Trematoda: Brachylaemidae). Morphological features of *Brachylaemus virginiana* metacercariae and migration route of cercariae in the second intermediate host. Anat. Record 111(3): 168.

WITENBERG, G.
1925. Versuch einer Monographie der Trematodenunterfamilie Harmostominae Braun. Zool. Jahrb., Jena. Abt. Syst. 51: 167–254.

DIFFERENTIAL EQUATIONS IN THE DISTRIBUTIONS OF SCHWARTZ

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This paper is an investigation in the recently developed distribution theory of Schwartz[1]. The notation used follows that of Schwartz. The value of the distribution T for the testing function ϕ will be denoted by $T.\phi$.

A. The Ordinary Linear Differential Equation.

Definition 1. The equation

$$p_0(x)T^{(n)} + p_1(x)T^{(n-1)} + \ldots + p_n(x)T = S,$$

where each $p_i(x)$ is indefinitely differentiable and S is a given distribution, is a linear differential equation of order n in distributions of one variable. If $S = 0$ the equation is homogeneous. If $p_0(x)$ has no zeros the equation is regular. The left side of the equation will be designated by $L(T)$, where L is the usual linear differential operator.

Theorem 1. $L(T).\phi = T.\bar{L}(\phi)$, where $\bar{L}$ is the operator adjoint to L.

Proof:
$$L(T).\phi = \Big(\sum_{i=0}^{n} p_i(x)T^{(n-i)}\Big).\phi$$
$$= T.\Big(\sum_{i=0}^{n} (-1)^{n-i}(p_i\phi)^{(n-i)}\Big)$$
$$= T.\bar{L}(\phi)$$

Theorem 2. The operator $\bar{L}$ maps the space of testing functions D onto D_α, a linear subspace of D. The mapping is continuous in the sense of D.

Proof: The proof that D_α is a linear subspace follows from the linearity of $\bar{L}$. Furthermore, consider a sequence of testing functions $\{\phi_j\}$. The continuity of the mapping means that if $\phi_j \longrightarrow 0$ in the sense of D, so also does $\bar{L}(\phi_j)$. Since each term in $\bar{L}(\phi_j)$ is the product of a continuous function with a derivative of ϕ_j, it is clear that the supports of the $\bar{L}(\phi_j)$ are contained in those of the ϕ_j. Also, if each sequence $\{\phi_j^{(k)}\}$ converges to 0 uniformly in x, the same is true for each sequence $\{L(\phi_j)^{(k)}\}$. This completes the proof.

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[1] Schwartz, Laurent. Theorie des Distributions. 2 Vols. Actualités Scientifiques et Industrielles. 1091, 1122. 1950, 1951.

Theorem 3. (a) If T_1 and T_2 are solutions of $L(T) = 0$, then $C_1T_1+C_2T_2$ is a solution, where C_1 and C_2 are arbitrary constants. (b) If T_1 and T_2 are solutions of $L(T) = S$, then their difference is a solution of $L(T) = 0$. (c) The totality of solutions of $L(T) = 0$ forms a linear subspace D'_α of the space of distributions D'. D'_α is the annihilator of D_α.

Proof: Parts (a) and (b) follow from the linearity of the equation. Part (c) follows from (a) and Theorem 1.

Theorem 4. If (a) the mapping $\bar{L}(\phi) = \alpha$ has a continuous inverse $\bar{L}^{-1}(\alpha) = \phi$, (b) the distributions $T_1, T_2, \ldots, T_n$ are a basis for the subspace of solutions of $L(T) = 0$, and (c) $T_1.\phi = T_2.\phi = \ldots = T_n.\phi = 0$ implies that ϕ is an element of D_α; then the differential equation $L(T) = S$ admits a unique general solution having the form

$$T.\phi = \sum_{i=1}^{n} C_iT_i.\phi + S.L^{-1}(\phi - \sum_{i=1}^{n} c_i\phi_i),$$

where $c_i = T_i.\phi$, and the ϕ_i are a linearly independent set of testing functions satisfying $T_i.\phi_j = \delta_{ij}$.

Proof: For any testing function θ in D,

$$L(T).\theta = T.\bar{L}(\theta) = S.\theta$$

by Theorem 1. This defines T uniquely on the subspace D_α, whose elements are $\alpha = \bar{L}(\theta)$, provided that if a sequence $\{\alpha_j\}$ approaches 0 in the sense of D, the corresponding sequence $\{\theta_j\}$ does also. Thus the reason for hypothesis (a) is evident. Now a set of linearly independent testing function $\phi_1, \phi_2, \ldots, \phi_n$ can be found which satisfy $T_i.\phi_j = \delta_{ij}$, by a well known algebraic theorem.[2] Therefore an arbitrary ϕ in D can be represented as

$$\phi = \sum_{i=1}^{n} c_i\phi_i + \alpha$$

by taking $c_i = T_i.\phi$. For the equations

$$T_j.\alpha = T_j.\phi - \sum_{i=1}^{n} c_iT_j.\phi_i$$

$$= T_j.\phi - c_j = 0, \quad j = 1,2,\ldots,n,$$

show that α is in D_α by hypothesis (c). Therefore a solution of $L(T) = S$ should satisfy

$$T.\phi = \sum_{i=1}^{n} c_iT.\phi_i + T.\alpha$$

$$= \sum_{i=1}^{n} C_iT_i.\phi + S.\theta.$$

Here the numbers $T.\phi_i$ are arbitrarily chosen and are represented by the constants C_i. The sum is seen to be the general solution of the homogeneous equation $L(T) = 0$ (Theorem 3). Substituting $\theta = \bar{L}^{-1}(\alpha)$ one gets the

[2] Lefschetz, S. Algebraic Topology, New York, Americal Mathematical Society, 1942, p.76 (25.9).

desired result. Finally it can be shown by a straightforward method that the above solution satisfies the linearity and continuity conditions for distributions. Examples can be given where the mapping defined by $\bar{L}$ has no inverse, or where the inverse is not continuous.

Consider the equation

$$xT' - \lambda T = S. \tag{1}$$

For this differential equation the operator L gives the corresponding equation in testing functions

$$\bar{L}(\theta) = -x\theta' - (1 + \lambda)\theta = \alpha, \tag{2}$$

which has the solution, for $-1 < \lambda < \infty$,

$$\theta(x) = -x^{-1}|x|^{-\lambda} \int_{-\infty}^{x} |t|^{\lambda}\, \alpha(t)dt + Cx^{-1}|x|^{-\lambda}. \tag{3}$$

But $\theta(x)$ must be a testing function and must therefore vanish identically for $|x|$ sufficiently large and be definable at $x = 0$. Necessary conditions to insure this are $C = 0$, and

$$\begin{aligned} T_1.\alpha &= \int_{-\infty}^{\infty} |t|^{\lambda}\, \alpha(t)dt = 0, \\ T_2.\alpha &= \int_{-\infty}^{0} |t|^{\lambda}\, \alpha(t)dt = 0. \end{aligned} \tag{4}$$

It can be shown that the above conditions are also sufficient, when $\theta(0)$ is defined to be $\lim_{x \to 0} \theta(x)$. The complete solution of (1) is therefore

$$T.\phi = C_1 T_1.\phi + C_2 T_2.\phi + S.\theta, \tag{5}$$

Where θ is given by (3) (with C equal to 0) and α is defined as in Theorem 4.

For $\lambda < -1$, equation (3) does not have meaning for all x. In the case where λ is not a negative integer one can take $-\lambda = \gamma + p$ (p is a positive integer and $0 < \gamma < 1$) and itegrate (3) by parts p times. Then $\theta(x)$ will be represented by

$$\theta(x) = -\left[\frac{\alpha(x)}{\lambda+1} - \frac{x\alpha'(x)}{(\lambda+1)(\lambda+2)} + \cdots + \frac{(-1)^p x^{p-1}\alpha^{(p-1)}(x)}{(\lambda+1)\cdots(\lambda+p)}\right. \tag{6}$$

$$\left. + \frac{(-1)^p |x|^{-\lambda} x^{-1}}{(\lambda+1)\cdots(\lambda+p)} \int_{-\infty}^{x} |t|^{-\gamma} \alpha^{(p)}(t)(\operatorname{sign} x)^p dt. \right]$$

It can be shown that necessary and sufficient conditions for θ as given by (5) to be a testing function are

$$\begin{aligned} T_1.\alpha &= \int_{-\infty}^{\infty} |t|^{-\gamma} \alpha^{(p)}(t)dt = FP \int_{-\infty}^{\infty} |t|^{-\lambda} \alpha^{(p)}(t)dt = 0, \\ \text{and} \quad T_2.\alpha &= \int_{-\infty}^{0} |t|^{-\gamma} \alpha^{(p)}(t)dt = FP \int_{-\infty}^{0} |t|^{-\lambda} \alpha^{(p)}(t)dt = 0. \end{aligned} \tag{7}$$

where FP denotes the finite part of an integral.[1]

For negative integral values of λ the same prodedure gives

$$\theta(x) = \frac{a(x)}{k-1} + \frac{xa'(x)}{(k-1)(k-2)} + \cdots + \frac{x^{k-2}a^{(k-2)}(x)}{(k-1)!} \tag{8}$$

$$- \frac{x^{k-1}\ln|x|\, a^{(k-1)}(x)}{(k-1)!} + \frac{x^{k-1}}{(k-1)!} \int_{-\infty}^{x} \ln|t|\, a^{(k)}(t)dt. \qquad (k = -\lambda)$$

Necessary and sufficient conditions for θ to be in D in this case are

$$T_1.a = \delta.a^{(k-1)} = 0, \text{ and}$$
$$T_2.a = \int_{-\infty}^{\infty} \ln|t|\, a^{(k)}(t)dt = FP \int_{-\infty}^{\infty} t^{-k}\, a(t)dt = 0. \tag{9}$$

In the definition of T_1, $\delta.\phi = \phi(0)$ defines the Dirac distribution.

Thus the solution of (1) is given by (5) for all values of λ, $\theta(x)$ being given by equations (3), (6), and (8) for the respective cases, and the distributions T_1 and T_2 by equations (4), (7), and (9).

As an illustration of the above solution, take $\lambda = -1$ and $S = \delta$. The solution can easily be calculated from the general form. One gets

$$T.\phi = C_1 \delta.\phi + C_2(FP\ \frac{1}{x})\ .\phi + \int_{-\infty}^{\infty} \ln|x|\, \phi'(x)dx. \tag{10}$$

The last term, which corresponds to the particular integral of a differential equation in functions, is seen to be the 'psuedo-function' distribution corresponding to the function whose value is $-\frac{1}{x}$ for $x < 0$, and 0 otherwise. It may also be represented as

$$FP \int_{-\infty}^{\infty} -1/x\ \phi(x)dx.$$

Using the same methods[3], solutions have been obtained for the equations (a) $T^{(n)} = S$, (b) $T' + f(x)T = S$, (c) $x^2T' + T = S$, (d) $x^3T' + 2T = S$, (e) $x(x-1)T' + T = S$, (f) $x^2T'' + xT' = S$, (g) $\sin xT' + \cos xT = S$.

B. The Heat Equation as a Partial Differential Equation in Distributions.

A solution of the 'equation of composition'

$$L(\delta) * T = \delta, \tag{11}$$

where L is a linear differential operator with constant coefficients, is an elementary solution of the more general equation

$$L(\delta) * T = S. \tag{12}$$

Solutions of (12) may be obtained from the elementary solution by means of

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[3]Gates, L. D., Differential Equations in the Distributions of Schwartz, Unpublished Ph.D. thesis, Ames, Iowa, Iowa State College Library, 1952.

a theorem due to Schwartz.[1] The method, as will be shown, justifies techniques previously used in connection with the heat equation, especially where the description is based on the so-called Dirac delta 'function'.

Theorem 5. If the distribution E satisfies $L(\delta) * E = \delta$, then the distribution $E * B$ satisfies $L(\delta) * (E * B) = B$, where B is a given distribution and L is a given operator.

The equation of conduction of heat in an isotropic homogeneous solid, in which heat is supplied at each point at the rate A(x,y,z,t) per unit time per unit volume, is known to be

$$\frac{\partial U}{\partial t} = k \nabla^2 U + A/\rho c, \tag{13}$$

where k is the diffusivity, ρ the density, and c the specific heat of the solid. For the sake of convenience $A/\rho c$ will be replaced by S, which will be considered to be a given distribution, the heat source. This gives the linear partial differential equation in distributions

$$k \nabla^2 U - \frac{\partial U}{\partial t} = -S. \tag{14}$$

Equation (14) may also be written

$$\left(k \nabla^2 \delta - \frac{\partial \delta}{\partial t}\right) * U = -S, \tag{15}$$

which has the form of an equation of composition.

The classical fundamental solution of the heat equation in its one-dimensional form is

$$V(x,t) = (4\pi kt)^{-\frac{1}{2}} \exp(-x^2/4kt), \tag{16}$$

which gives the temperature at any point and at any time after the introduction of a unit quantity of heat at x = 0 and t = 0. Thus V(x,t) should be representable as a distribution, the solution to

$$L(\delta) * U = -\delta, \tag{17}$$

where L is the operator in (15). The required V is given by

$$V.\phi(x,t) = \int_{-\infty}^{\infty}\int_{0}^{\infty} V(x,t)\phi(x,t)\,dt\,dx, \tag{18}$$

and it can be shown that

$$\begin{aligned}(L(\delta) * V).\phi &= \int_{-\infty}^{\infty}\int_{0}^{\infty} V(x,t)\left(k \frac{\partial^2 \phi}{\partial x^2} + \frac{\partial \phi}{\partial t}\right) dx\,dt \\ &= -\phi(0,0) = -\delta.\phi\end{aligned} \tag{19}$$

From this solution, other solutions can be derived for given S, by the method of Theorem 5. For example, if the source S is given by

$$S.\phi = (\delta_t \times \delta'_x).\phi = -\frac{\partial \phi(x,t)}{\partial x}\Bigg]_{\substack{x=x_0 \\ t=0}}, \tag{20}$$

then S corresponds to an instantaneous point doublet at $x = x_0$, $t = 0$.

One then calculates

$$(S * V).\phi = -\frac{\partial}{\partial x'} \int_{-\infty}^{\infty}\int_{0}^{\infty} V(x,t)\phi(x+x',\ t+t')\ dtdx. \Big|_{\substack{x'=x_0 \\ t'=0}} \qquad (21)$$

After performing the differentiation under the integral sign, integrating by parts and replacing x by $x - x_0$, one gets

$$(S * V).\phi = U.\phi = \int_{-\infty}^{\infty}\int_{0}^{\infty} f(x,t)\phi(x,t)dtdx,$$

where

$$f(x,t) = \frac{x - x_0}{2kt(4\pi kt)^{\frac{1}{2}}} \exp \frac{-(x - x_0)^2}{4kt}. \qquad (22)$$

Other such derived solutions have been calculated,[3] and have been found to agree with results from classical literature.[4]

In the classical study of boundary value problems, the method of solution by separation of variables is of great importance. It has been found possible to adopt this method for use in distributional equations, as the following illustration will show.

In equation (14), let S be given by $S = 1_t \times \delta_x$, where the delta distribution is at $x = x_0$. S is therefore a 'distribution of one variable' and we will abbreviate the notation by writing $S = \delta_x$. Now if the assumption is made that $U = W + 1_t \times R_x$, (14) becomes

$$k\,\frac{\partial^2 W}{\partial x^2} - \frac{\partial W}{\partial t} + k\,\frac{d^2 R_x}{dx^2} = -\delta_x . \qquad (23)$$

Equation (23) will be satisfied if

$$k\,\frac{d^2 R_x}{dx^2} = -\delta_x, \text{ and} \qquad (24)$$

$$k\,\frac{\partial^2 W}{\partial x^2} - \frac{\partial W}{\partial t} = 0. \qquad (25)$$

The solution of (24) can be shown to be

$$R_x \longleftrightarrow C_1 x + C_2 + g(x),$$

$$g(x) = \begin{cases} 0 & , -\infty < x < x_0 \\ (x_0 - x)/k, & x_0 < x < \infty . \end{cases} \qquad (26)$$

In (26) the double arrow means that the distribution corresponds to the locally summable function on the right. Now assume in (25) that $W = X_x \times T_t$ and calculate $W.\phi$, where $\phi(x,t) = u(x)v(t)$. This gives

$$kW.(u''v) = -W.(uv'),$$

or

$$k(X.u'')(T.v) = -(X.u)(T.v'). \qquad (27)$$

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[4] Carslaw, H. S., and Jaeger, J. C., Conduction of Heat in Solids, Oxford, Oxford University Press, 1947, Chap. 10.

Equation (27) must hold for a fixed u and arbitrary v or vice-versa. Thus for any u and v

$$k\,\frac{X.u''}{X.u} = -\frac{T.v'}{T.v} = -\lambda\ , \tag{28}$$

and one is justified in saying that the distributions X and T must satisfy the differential equations

$$\begin{aligned} X'' + \lambda/k\, X &= 0, \\ T' + \lambda\, T &= 0. \end{aligned} \tag{29}$$

The latter are readily solved and the complete solution is seen to be

$$\begin{aligned} U \longleftrightarrow U(x,t) = (A \sin \sqrt{\lambda/k}\, x + B \cos \sqrt{\lambda/k}\, x)e^{-\lambda t} \\ + C_1 x + C_2 + g(x), \end{aligned} \tag{30}$$

where A, B, and C_i are arbitrary constants.

Problems involving specified boundary conditions enabling the constants in (30) to be determined have been considered.[3] For this purpose the value of a distribution at a point is defined to be the limit of the sequence $\{T.\phi_j\}$, where $\{\phi_j\}$ is a sequence of positive testing functions whose supports are $x_0 - 1/j < x < x_0 + 1/j$ and which satisfy $\int_{-\infty}^{\infty} \phi_j(x)dx = 1$, where the limit exists.